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Resolving the genetic structure of striped marlin, *Kajikia audax*, in the Pacific Ocean through spatial and temporal sampling of adult and immature fish

Catherine M. Purcell and Suzanne Edmands

Abstract: Spatial genetic structure in the highly migratory striped marlin (*Kajikia audax*) was examined using nuclear (microsatellite) and mitochondrial (control region sequences) DNA markers. While previous studies on striped marlin were limited by sampling design and sample size, this study employed a multiyear concurrent sampling scheme to collect tissue from seven locations representative of the species' range in the Pacific: Japan, Hawaii, Southern California, Mexico, Central America, New Zealand, and Australia. Mature and immature specimens were analyzed separately to evaluate life-stage-specific population structure and movements. Microsatellite and sequence results revealed small but significant overall spatial subdivision (F_{ST} = 0.0145 and K_{ST} = 0.06995, respectively). Pairwise microsatellite analyses (n = 1199) revealed four groups: (1) Japan – Immature Hawaii – Southern California, (2) Mature Hawaii, (3) Mexico – Central America, and (4) Australia – New Zealand. Mitochondrial sequence analysis (n = 451) showed similar patterns; however, no significant differentiation was found between groups 1 and 2. This enhanced resolution of geographic genetic structure is important for understanding the complex migration patterns in this species. Moreover, the consistency among independent genetic studies on striped marlin provides strong support for management of at least three clearly delineated Pacific stocks.

Résumé : Des marqueurs nucléaires (microsatellites) et mitochondriaux (séquences de la région de contrôle) d'ADN nous ont servi à déterminer la structure génétique spatiale du makaire strié (*Kajikia audax*), une espèce fortement migratrice. Alors que les études antérieures sur le makaire strié ont été limitées par leur plan d'échantillonnage et la taille des échantillons, notre étude utilise une méthode d'échantillonnage concurrent sur plusieurs années pour récolter des tissus dans sept régions représentatives de l'aire de répartition de l'espèce dans le Pacifique, soit le Japon, Hawaii, le sud de la Californie, le Mexique, l'Amérique Centrale, la Nouvelle-Zélande et l'Australie. Nous avons analysé les spécimens matures et immatures séparément de manière à évaluer la structure génétique et les déplacements spécifiques au stade de vie. Les résultats des analyses des microsatellites et des séquences indiquent globalement une subdivision faible mais significative (respectivement, F_{ST} = 0,0145 et K_{ST} = 0,06995). Des analyses appariées des microsatellites (n = 1199) révèlent l'existence de quatre groupes : (1) Japon – immatures d'Hawaii – sud de la Californie, (2) matures d'Hawaii, (3) Mexique – Amérique Centrale et (4) Australie – Nouvelle-Zélande. L'analyse des séquences mitochondriales (n = 451) montre des patrons similaires; il n'existe cependant pas de différenciation significative entre les groupes 1 et 2. Cette différenciation améliorée de la structure génétique géographique est importante pour comprendre les patrons complexes de migration de l'espèce. De plus, la concordance entre les études génétiques du makaire strié appuient fortement une gestion basée sur au moins trois stocks bien définis dans le Pacifique.

[Traduit par la Rédaction]

Introduction

Large pelagic species such as tuna and billfish roam the world's oceans, free of any obvious physical barriers. It has generally been thought that the highly migratory lifestyles of these marine fishes would lead to genetic homogeneity among conspecific populations, yet molecular data show that this is not always true. While some of these free-roaming fish show no genetic structure between or within oceans (e.g., skipjack tuna (*Katsuwonus pelamis*) and wahoo (*Acanthocybium solandri*) (Ely et al. 2005; Theisen et al. 2008), other species exhibit varying levels of genetic subdivision, such as blue marlin (*Makaira nigricans*) (Finnerty and Block 1992; Buonaccorsi and Graves 2000; Buonaccorsi et al. 2001) and bigeye tuna (*Thunnus obesus*) (Durand et al. 2005), that show genetic differentiation between Atlantic and Pacific Ocean populations. Genetic subdivision has also been seen within ocean basins; swordfish (*Xiphias gladius*) (Block and Reeb 2000), Pacific sailfish (*Istiophorus platypterus*) (Graves and McDowell 1995), and striped marlin (*Kajikia audax*) (Graves and McDowell 1994; McDowell and Graves 2008) all revealed subdivision within the Pacific Ocean.

While generally considered a bycatch species, striped marlin are sold commercially in Japan, Taiwan, Australia, and Hawaii, with smaller targeted fisheries in several locations. Striped marlin are also important to recreational fisheries around the Pacific, particularly in Hawaii, New Zealand, Aus-

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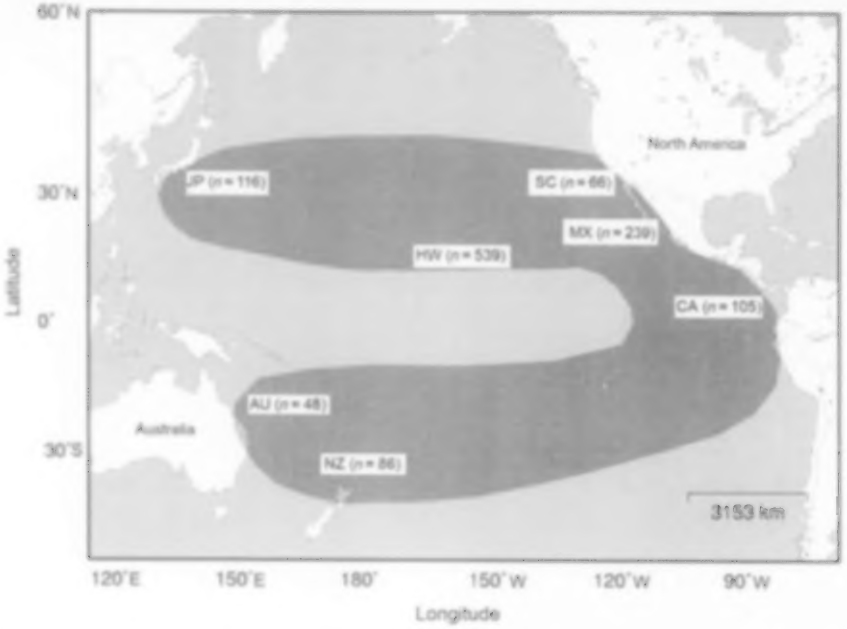
Fig. 1. The Pacific Ocean range of striped marlin (*Kajikia audax*) (shaded area), sampling locations, and number of specimens collected in those locations.



tralia, Southern California, and Mexico. These recreational fisheries contribute greatly to coastal economies through sportfishing and sportfishing-related tourism (Bromhead et al. 2004). Given this species' economical importance and the signs of fishing-related population strain already occurring (Bromhead et al. 2004; Worm et al. 2005), it is important to understand its spatial distribution to develop effective management strategies. This study focuses on examining the spatial genetic structure of striped marlin in the Pacific Ocean.

Previous work

Striped marlin occurs in the Pacific and Indian oceans and is considered the most abundant and widely distributed billfish (Nakamura 1985). In the Pacific, its distribution creates a horseshoe-shaped pattern across the ocean basin (Fig. 1), occurring in tropical, subtropical, and temperate regions. Individual striped marlin are capable of moving throughout their range as evidenced by tagging studies; however, the northernmost and southernmost extensions of their range are seasonal, as the waters become too cool during winter months (Squire 1972).

Several stock scenarios have been proposed for striped marlin: a single panmictic population within the Pacific (Shomura 1980), eastern–western Pacific stocks (Morrow 1957), and northern–southern Pacific stocks (Kamimura and Honma 1958). Alternatively, a regional stock hypothesis is bolstered by spatial and temporal partitioning of spawning events and may also fit with the results of Graves and McDowell (1994), who used mitochondrial restriction fragment length polymorphisms (RFLPs) in four Pacific locations to find shallow but significant levels of genetic subdivision, with 4/6 significant pairwise comparisons. These findings were again supported by a study using five microsatellites and mitochondrial control region sequences (McDowell and Graves 2008); the results suggested four regional stocks: Southwest Pacific (Australia), North Pacific (Japan–Taiwan–Hawaii–California), Mexico, and Southeast Pacific (Ecuador).

Despite previous investigations into the striped marlin's population structure, questions remained regarding the applicability of these studies to management issues because of sampling design, temporal replicates, and number of specimens. McDowell and Graves (2008) attempted to address these issues from their first study (Graves and McDowell 1994); however, with the complexities associated with sampling large pelagic fish, some of these difficulties persisted. Sample size, for instance, may have continued to be a limitation to the 2008 study; while 371 specimens were screened with microsatellites, only 85 specimens (from six of the seven locations) were sequenced at the mitochondrial control region. Pairwise mtDNA estimates did not detect genetic subdivision, possibly because of small sample size. These sampling limitations make it difficult to apply results of previous studies to striped marlin management issues.

In this project, sampling was conducted over seven locations representative of the striped marlin's range in the Pacific, with concurrently sampled temporal replicates in six of the locations. Unlike previous billfish studies, to better assess where breeding populations occur, initial analyses kept mature and immature individuals (in applicable locations) separate to determine if they were significantly different. To increase resolution power, two classes of molecular markers were used: microsatellites (12 loci) and mitochondrial DNA control region sequences. Concordance among markers adds confidence in the findings; however, differences among markers can also be informative and can help distinguish genetic signals due to selection or other forces.

Given that genetic variability in striped marlin is high and that sample size can greatly influence the patterns of genetic subdivision, a total of 1199 specimens were screened with microsatellites and 451 specimens were sequenced at the mtDNA control region. A greater number of loci, larger sample sizes, and a concurrent sampling scheme may more conclusively resolve population subdivision than previous studies and increase the usefulness of these results to managing agencies. Additionally, temporal analyses of the same data set (Purcell 2009; C. Purcell and S. Edmands, unpublished data) show that the spatial subdivision patterns reported here are largely robust to temporal variance.

While genetic subdivision is difficult to detect in migratory species, as even a few migrants would erase patterns of subdivision, the variation observed in striped marlin suggests differences in life-history traits and (or) population demographics between this species and the other migratory species not exhibiting genetic structure. Trying to understand the cause of these differences is crucial in developing effective management strategies to preserve this fishery.

Materials and methods

Sampling strategy

From 2001 to 2007, samples were collected from seven locations chosen to be representative of the species' range in the Pacific: Japan (2003), Hawaii (2003, 2004, 2005), Southern California (2001, 2002, 2004, 2005, 2006, 2007), Mexico (2003, 2004, 2005, 2006, 2007), Central America (2003, 2004, 2005, 2006), New Zealand (2004, 2006), and Australia (2004, 2005) (Fig. 1). The samples were provided through commercial and recreational fishing efforts. Commercial samples were collected through sponsored observer programs in collaboration with the National Marine Fisheries Service (NMFS), the Inter-American Tropical Tuna Commission (IATTC), the National Research Institute of the Far Seas Fisheries (NRIFSF, Japan), and the Secretariat for the Pacific Community (SPC, New Caledonia). Recreational samples were collected through independent environmental research firms, such as the Pfleger Institute of Environmental Research (Oceanside, California), Marine Conservation Science Institute (Fallbrook, California), Pepperell Research and Consulting (New South Wales, Australia), and Nelson Resources Consulting (Miami, Florida), and scientists working with Interdisciplinario de Ciencias Marinas (CICIMAR, Mexico). Recreational fishers also assisted in providing samples from kill tournaments and from live fish on catch-and-release trips with biopsy darts mounted on tagging poles. Sample numbers varied by location because of the overall abundance of striped marlin in the area, the type of fishing used to obtain samples, and the participation of contacts in those areas. Fin or muscle tissue was preserved in ethanol or 20% dimethyl sulfoxide buffer saturated with sodium chloride (Seutin et al. 1991). Some samples were not used in genetic analyses because of tissue degradation.

Sample preparation

For higher quality tissue, genomic DNA was extracted from small amounts of tissue using Chelex (BioRad) or lysis reactions (Edmands et al. 2005). In the lysis extraction, a few muscle tissue fibers or a small amount of fin skin were added to 50 µL lysis buffer at 65 °C for 1 h followed by 100 °C for 15 min. For degraded samples, an overnight cetyltrimethylammonium bromide – proteinase K incubation was followed by a standard phenol–chloroform extraction, with an ethanol precipitation, and if necessary a lithium chloride wash.

Determination of maturity

Samples were divided into reproductively immature and mature individuals using several methods because of variable biological information collected from the sample sources. Based on fish in the Coral Sea (Hanamoto 1977), 29 kg was used as the mass at first maturity and 143 cm was used as eye fork length at first maturity. A factor of 1.2 (dressed mass × 1.2 = round mass) was used to convert dressed masses into round masses based on published International Convention for the Conservation of Atlantic Tunas (ICCAT) estimates for billfish (Mejuto et al. 2002). Lower jaw fork lengths were converted into masses based on Kopf et al. (2005).

Microsatellite assays

Twelve microsatellite loci were used in this study: ten developed specifically for this project as described by Purcell et al. (2009) and two developed by Buonaccorsi and Graves (2000). Polymerase chain reaction (PCR) amplification conditions varied between microsatellite primer sets. Five microsatellites were amplified using specific fluorescently labeled forward primers; the other seven microsatellites were amplified with a modified nonlabeled forward primer containing a 25 bp zip-code tag (Chen et al. 2000). Fluorescent complementary primers for the zip-code tags were used for amplification of those modified microsatellites, with the resulting fragment sizes 25 bp longer. Both sets of fluorescent primers used Beckman WellRED D2, D3, or D4 dyes. PCR was conducted on a MJ Research PTC-200 DNA Engine and an Applied Biosystems GeneAmp PCR System 9700 with the following conditions: 15 ng template DNA, 0.25–1.0 µmol·L^{-1} primers, 1.5–3.5 mmol·L^{-1} $MgCl_2$, 0.25 mmol·L^{-1} dNTPs, 10 mmol·L^{-1} Tris-HCl, 50 mmol·L^{-1} KCl, and 0.3 U *Taq* polymerase in 12 µL total volume. Two cycling conditions were used: one for specifically labeled forward primers and another for labeled zip-code tags, described in Purcell et al. (2009). PCR products were analyzed using the fragment analysis on a Beckman–Coulter CEQ 8000 Capillary Sequencer and scored visually. Approximately 7% of samples were rerun for consistency in PCR amplification and fragment analysis on the sequencer. For scoring consistency of microsatellite fragments, approximately 20% of samples were rescored.

Mitochondrial control region assays

The mitochondrial control region was amplified for 451 striped marlin specimens from the locations listed above, with five specimens from Ecuador used in two of the analyses. PCR was conducted using three universal primers (K: 5′-AGCTCAGCGCCAGAGCGCCGGTCTTGTAAA-3′) (Lee et al. 1995), (L19: 5′-CCACTAGCTCCCAAAGCTA-3′) (Bernatchez et al. 1992), and (12 SAR-H: 5′-ATAGTGGGGTATCTAATCCCAGTT-3′) (Palumbi et al. 1991) to amplify approximately 1000 bp of this region. PCR was conducted with the following conditions: 25 ng template DNA,

1 μmol·L^{-1} forward and reverse primers, 0.25 mmol·L^{-1} dNTPs, 2.0–2.5 mmol·L^{-1} $MgCl_2$, 10 mmol·L^{-1} Tris–HCl, 50 mmol·L^{-1} KCl, and 1 U *Taq* polymerase in a 34 μL total volume. The cycling conditions consisted of the following: initial denaturation at 93 °C for 5 min, 35 cycles of denaturation at 93 °C for 1 min, primer annealing at 54 °C for 30 s, followed by extension at 72 °C for 1 min 15 s. After 35 cycles, a 5 min final extension at 72 °C was used, followed by a hold at 8 °C. After checking for amplification on 1.2% agarose gels, successful amplifications were submitted to High-Throughput Sequencing Solutions operated by the University of Washington, Department of Genome Sciences (Seattle, Washington) for ExoSAP PCR clean-up and sequencing using the primers from the original amplification. The entire control region was sequenced in both directions using those primers. Sequences were examined and aligned using SEQUENCHER 4.6 (Gene Codes Corporation).

Microsatellite data analyses

To explore spatial patterns in striped marlin, the 1199 specimens were first arranged by collection location and then further separated into mature and immature fish within each location (where applicable). For each population, observed (H_o) and expected (H_e) levels of heterozygosity were calculated using ARLEQUIN 3.1 (Excoffier et al. 2005). Deviations from Hardy–Weinberg equilibrium (HWE) were detected using F_{IS}, and genotypic disequilibrium was calculated for each locus-pair in each population using GENEPOP 1.2 (Raymond and Rousset 1995) with 10 000 dememorization steps, 1000 batches, and 10 000 iterations. The program ML-NullFreq (Kalinowski et al. 2006) was used to check for null allele frequency in each locus and population. The original data set was corrected for null alleles based on estimated frequencies found using ML-NullFreq, and the expected number of null homozygotes and heterozygotes were calculated for all loci within each population. The expected numbers of null homozygotes were added to individuals with missing data using the null allele "999". Null heterozygotes were incorporated by randomly adding the null allele to existing non-null allele homozygotes. The data incorporating the null alleles were permutated to mimic a round of sexual reproduction, thereby randomly mixing the null alleles throughout the population using GENETIX 4.04 (Belkir et al. 2000).

Using null-corrected data, the genetic structure of mature and immature groups within locations was determined. If no significant differences were found between mature and immature samples in a location, they were combined for the remaining analyses. However, if groups showed significant structure, then they remained as separate groups for the rest of the analyses.

Because the null allele correction method permutated data within locations to shuffle the null alleles, the original (non-corrected) data from the sample groups determined above were used to estimate observed and expected heterozygosity, F_{IS}, and genotypic disequilibrium using the programs and parameters mentioned above. Genetic variability among–within striped marlin populations, measured by allelic richness (A_R) and the number of alleles (N_A), was also calculated with the non-null-corrected data using FSTAT 2.9.3 (Goudet 1995).

Weir and Cockerham's overall F_{ST} and pairwise F_{ST} values were calculated for all sample groups using 10 000 permutations in GENETIX 4.04 (Belkir et al. 2000), followed by a straight Bonferroni correction of the pairwise estimates. For comparison, this was conducted for null-corrected and non-corrected data. Hedrick's G'_{ST} (2005) was calculated from estimates of Nei and Chesser's (1983) G_{ST} and H_S given in GENETIX (Belkir et al. 2000) for the null-corrected data. The program ISOLDE within GENEPOP was used to test the correlation between geographic distance, measured as the average distance among sampling locations in kilometres (km) and population structure, as estimated with F_{ST}. Correlation significance between genetic structure and geographical distance was assessed using the Mantel test in GENEPOP with 1000 permutations.

The model-based Bayesian clustering program STRUCTURE 2.2 (Pritchard et al. 2000) was also used to examine population structure. Five replicates were run with a burn-in of 100 000 steps and 500 000 Markov chain Monte Carlo (MCMC) steps for K from 1 to 11, using the admixture model and the assumption that allele frequencies are correlated among populations.

Mitochondrial data analyses

The number of haplotypes (h), haplotype diversity (h_d), and average number of differences between sequences (K) were calculated for sequences in each location using DNASP 4.50.3 (Rozas et al. 2003). The haplotype-based statistic (H_{ST}) and nucleotide sequence-based statistic (K_{ST}) were calculated in DNASP. Population pairwise comparisons were conducted for the average number of pairwise nucleotide differences (K_{xy}) and for the nearest-neighbor statistic, S_{nn}, which is a measure of how often the most similar sequences are from the same location (Hudson 2000) in DNASP. Also in DNASP, pairwise genetic subdivision estimates were calculated using the sequence-based statistic (K_{ST}) according to Hudson et al. (1992). A neighbor-joining tree was created using ClustalX 2.0.10 (Larkin et al. 2007) and MEGA 4 (Tamura et al. 2007) using a bootstrap test with 5000 replicates. A hierarchical structuring of the mitochondrial sequences using an analysis of molecular variance (AMOVA) within ARLEQUIN was used to assess the relative contribution of variance among groups, within groups, and within populations using the distance matrix of pairwise differences among sequences.

Results

Microsatellite analyses

During the microsatellite analysis, initial comparisons of observed and expected heterozygosities, F_{IS} values, and null allele frequency estimates indicated that a null allele correction should be incorporated into the data set (Supplemental Table S1[1], available online). Null frequencies ranged from 0% to 33.6% of homozygotes for a few locus–location combinations; however, it is important to note that even higher estimates may have reflected only one or two homozygous

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-104.

individuals. Following this correction, the data were used to compare the mature and immature specimens within the locations. Based on F_{ST} estimates, immature and mature Mexican specimens and immature and mature Central American specimens were grouped together for the remaining analyses. However, significant structure was detected between immature and mature Hawaiian specimens, and they were therefore maintained as separate groups.

Genetic variability (microsatellites)

Because the null allele correction permuted data within locations to randomly distribute null alleles among samples, the original data (not corrected) was used in all summary statistics. Genetic variability (Table 1) within striped marlin populations varied widely among loci; allelic richness ranged from 2.4 (locus *164*) to 7 (locus *Mn08*), both in Japan, but no significant differences were found among locations in allelic richness ($p = 0.99$, using a two-tailed test implemented in FSTAT with 1000 permutations). The number of alleles varied widely depending on the locus, from 2 alleles in Australia and New Zealand (locus *218*) to 49 alleles (locus *Mn08*) in Mature Hawaii and Mexico, but again was not significantly different among locations ($p = 0.54$, using a two-tailed test implemented in FSTAT with 1000 permutations).

Observed levels of heterozygosity (H_o) also varied greatly among microsatellite loci (Table 1); however, average heterozygosity across all loci for each location fell within a narrow range. Lowest average H_o (0.652) was in Southern California, and the highest averages (0.727 and 0.696) were in Australia and New Zealand, respectively; neither H_o nor H_e among locations were significantly different ($p = 0.98$ and $p = 0.99$, respectively). Significant heterozygote deficits were seen in Mature Hawaii (seven loci), Immature Hawaii (five loci), Australia (four loci), Mexico (three loci), New Zealand (two loci), and Southern California and Central America (one locus each). Japan showed no significant differences.

F_{IS} was also used to investigate deviations from HWE (Table 1), and tests were conducted for the eight population groups, including the two groups from Hawaii. Numbers ranged from positive values, indicating a deficit of heterozygotes, to negative values, indicating an excess of heterozygotes. Significant deviations were found in Japan (three loci), Mature Hawaii (nine loci), Immature Hawaii (four loci), Southern California (four loci), Mexico (four loci), Central America (three loci), New Zealand (one locus), and Australia (three loci) (Table 1). F_{IS} values were predominately positive, and 32 of the 33 significant values were positive, indicating a heterozygote deficit.

Genotypic disequilibrium for all locus-pairs was calculated for the eight sample groups (Supplemental Table S2[1]) using the log likelihood ratio statistic (G test) and the Markov chain method with the parameters 10 000 dememorization steps, 1000 batches, and 10 000 iterations in GENEPOP. Following Bonferroni correction, significant disequilibrium was detected in 37 locus-pairs in Australia and one locus-pair in Mature Hawaii (Supplemental Table S2[1]). In total, 56% of locus-pairs in Australia showed significant genotypic disequilibrium.

Genetic structure (microsatellites)

Spatial structure was calculated using Wright's F_{ST} according to Weir and Cockerham (1984) and Hedrick's standardized genetic differentiation estimate, G'_{ST}, at all loci for each population using GENETIX. Significance levels were assessed using 10 000 permutations. These estimates were conducted for both null-corrected and original data to examine effects of null alleles. Although F_{ST} values were small, they were significant for the null-corrected and original data sets, 0.0145 and 0.0123, respectively. As expected, Hedrick's G'_{ST} estimates for both groups were much larger than the F_{ST} estimates, 0.0802 (null-corrected data) and 0.0559 (original data).

Pairwise F_{ST} comparisons for the null-corrected data (Table 2) detected significant subdivision in 23 of 28 comparisons. The five sample pairs without significant differentiation were Japan – Immature Hawaii, Japan – Southern California, Immature Hawaii – Southern California, Mexico – Central America, and New Zealand – Australia. The values and range of values in pairwise comparisons were larger using G'_{ST} rather than F_{ST} estimates, with the largest value of G'_{ST}, 0.1345, found between Southern California and Australia, and the smallest, 0.0050, found between Japan and Southern California (Table 2). This is compared with the largest F_{ST} estimate, 0.0374, between Mexico and Australia, and the smallest, 0.0013, between Southern California and Japan (Table 2). Despite the spatial heterogeneity detected using the estimates above, analyses using STRUCTURE showed that a single population ($K = 1$) provided the best fit to the data with the largest log-likelihood estimate of –41 364.2.

Pairwise F_{ST} comparisons of the original data revealed similar patterns to the null-corrected analysis, but with fewer significant relationships (Table 2). Key differences were that the original data showed no significant pairwise differences between Mature Hawaii and the Immature Hawaii, Southern California, and New Zealand sample groups. The smallest F_{ST} value, 0.0002, was found between Japan and Immature Hawaii, and the largest, 0.0340, between Central America and New Zealand. Hedrick's G'_{ST} showed a wider range in estimates, from 0.0004 (Japan – Immature Hawaii) to 0.0771 (Mexico–Australia). A significant positive correlation was found between geographic distance (average distance among sampling locations) and population structure (F_{ST}) for striped marlin specimens from different locations (one-sided Mantel test with 1000 permutations, $p = 0.0087$, $R = 0.3559$). The scatterplot of the average distance among sampling locations (km) and F_{ST} is shown (Fig. 2).

Genetic variability (mitochondrial sequences)

A total of 451 individuals were sequenced (GenBank accession Nos. JF755428–JF755878) from eight locations: Japan, Hawaii, Southern California, Mexico, Ecuador ($n = 5$, not shown in Table 3), Central America, New Zealand, and Australia. Out of 451 control region sequences, 351 were unique haplotypes. Relative to the number of sequences, the number of haplotypes in each location was very high, ranging from 18 (Central America) to 91 (Mexico) (Table 3). Not surprisingly, haplotype diversity was very high among the locations (Table 3). The average number of differences between sequences (K) ranged from a low in Australia (26) to a high in Central America (42).

Genetic structure (mitochondrial sequences)

The overall genetic differentiation based on the mitochon-

Table 1. For each location, data is shown for the number of specimens analyzed with each microsatellite locus (n), allelic richness (A_R), number of alleles per locus (N_A), observed (H_o) and expected (H_e) levels of heterozygosity (H_o/H_e), and F_{IS} values used to detect deviation from the Hardy–Weinberg equilibrium.

	Microsatellite locus											
	24	*157*	*162*	*164*	*218*	*235*	*105*	*149*	*155*	*193*	*Mu61*	*Mu68*
JP (n = 119)												
n	110	110	108	111	108	108	61	76	68	85	43	50
A_R	5.5	3	5.1	2.4	2.5	5.4	6.2	2.8	3.6	6.3	5.2	7.2
N_A	19	5	17	8	4	20	19	5	8	26	13	34
H_o/H_e	0.882/0.881	0.491/0.558	0.756/0.817	0.396/0.383	0.481/0.510	0.870/0.869	0.902/0.923	0.400/0.443	0.448/0.599	0.906/0.925	0.744/0.821	0.898/0.968
F_{IS}	–0.001	0.121	0.071	–0.036	0.057**	–0.002*	0.023	0.098	0.254	0.021**	0.094	0.073
MT HW (n = 312)												
n	282	279	275	279	222	274	100	125	166	180	98	193
A_R	5.6	3.1	5.2	3.2	3	5.8	6.3	3.1	3	6.4	5.3	7
N_A	25	6	21	10	7	27	25	6	6	30	15	49
H_o/H_e	0.876/0.889	0.513/0.560***	0.763/0.832***	0.446/0.510	0.489/0.547***	0.813/0.888***	0.786/0.920*	0.333/0.403	0.333/0.423***	0.817/0.927	0.786/0.844	0.932/0.958***
F_{IS}	0.015	0.085**	0.084***	0.126	0.107***	0.084***	0.145***	0.173	0.212***	0.119***	0.069***	0.027*
IM HW (n = 227)												
n	196	197	201	207	172	204	128	144	120	157	76	125
A_R	5.5	2.6	5.3	3.3	2.6	5.4	6.4	2.2	3.5	6.6	5.3	7.1
N_A	26	6	19	9	5	21	30	4	8	41	15	40
H_o/H_e	0.857/0.880	0.538/0.552	0.735/0.826***	0.398/0.489	0.444/0.504*	0.853/0.874	0.754/0.927***	0.486/0.472	0.431/0.566***	0.821/0.935**	0.803/0.844	0.920/0.960
F_{IS}	0.026	0.026	0.110***	0.186	0.118**	0.024	0.187***	–0.03	0.240***	0.123***	0.049**	0.042
SC (n = 66)												
n	58	61	59	59	61	63	29	44	37	38	22	37
A_R	6.1	2.6	4.6	2.3	2.4	5.5	6.1	2.9	3.3	6.6	5.4	7
N_A	19	4	16	7	4	16	17	5	6	22	9	30
H_o/H_e	0.862/0.911	0.508/0.535	0.746/0.751	0.356/0.353	0.574/0.517	0.857/0.875	0.862/0.910	0.349/0.436	0.286/0.462	0.868/0.934	0.773/0.855	0.779/0.966*
F_{IS}	0.054*	0.051	0.007	–0.01	–0.111	0.021*	0.053	0.202	0.385	0.071*	0.098	0.197***
MX (n = 239)												
n	218	218	214	224	183	211	84	106	117	188	79	174
A_R	5.5	4.6	2.1	3	6.3	3.2	6.6	2.8	5.4	2.3	4.5	7
N_A	19	7	16	6	6	22	24	5	7	35	13	49
H_o/H_e	0.858/0.879	0.564/0.581	0.687/0.760	0.339/0.330	0.404/0.509***	0.806/0.856***	0.845/0.921	0.500/0.465	0.624/0.617	0.855/0.934	0.744/0.774	0.920/0.957*
F_{IS}	0.024	0.029	0.096	–0.027	0.205**	0.059***	0.083**	–0.076	–0.011	0.085**	0.039	0.04
CA (n = 105)												
n	100	99	104	103	93	102	31	60	48	68	37	64
A_R	5.6	4.9	2.3	3.4	5.9	3.9	6.5	2.3	5.2	2.9	4.7	7
N_A	17	6	16	7	3	16	16	7	9	28	9	37
H_o/H_e	0.890/0.884	0.485/0.567	0.680/0.783	0.427/0.383	0.462/0.503	0.824/0.847	0.667/0.907	0.417/0.458	0.604/0.674	0.897/0.933	0.794/0.782	0.794/0.963*
F_{IS}	–0.007	0.145	0.132	–0.115	0.081	0.027	0.269**	0.091	0.105	0.039*	–0.015	0.177***
NZ (n = 86)												
n	76	71	78	79	80	79	56	29	67	58	56	67
A_R	5.7	2.8	5.3	3.7	2	5.6	6.4	3.2	2.8	6.3	5	6.9
N_A	17	6	13	7	2	15	23	5	6	23	12	33
H_o/H_e	0.895/0.893	0.535/0.542	0.808/0.860	0.582/0.653	0.538/0.499	0.899/0.892	0.746/0.933*	0.517/0.542	0.349/0.403	0.772/0.920*	0.804/0.827	0.911/0.951
F_{IS}	–0.002	0.012	0.062	0.109	–0.077	–0.008	0.203***	0.047	0.137	0.162	0.029	0.043

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Table 1 (*concluded*).

	Microsatellite locus													
	24	*157*	*162*	*164*	*218*	*235*	*305*	*349*	*353*	*393*	*Mdh1*	*Mdh8*
AU (n = 48)												
n	42	44	41	45	43	45	42	44	44	44	42	42
A_R	5.1	2.5	5.4	4	2	4.9	6.3	2.1	3.4	6.4	4.5	6.6
N_A	14	4	13	9	2	9	21	3	6	20	9	22
H_O/H_E	0.85710.838	0.6360.556	0.67560.852**	0.71140.731	0.51250.499	0.82260.845	0.78660.929*	0.54560.484	0.61460.656	0.75060.926***	0.88180.862	0.92980.944***
F_{IS}	–0.023	–0.146	0.210*	0.027	–0.026	0.027	0.147*	–0.129	0.066	0.192**	–0.1	0.017

Note: Significant p values are denoted as follows: *, $P < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. JP, Japan; MT HW, Mature Hawaii; IM HW, Immature Hawaii; SC, Southern California; MX, Mexico; CA, Central America; NZ, New Zealand; AU, Australia.

drial sequences was calculated by two methods, H_{ST} and K_{ST}, that use the χ^2 statistical approach proposed by Hudson et al. (1992, eq. 2 and eq. 9, respectively). Both methods showed significant differentiation, with H_{ST}, the haplotype-based statistic estimated at 0.00493 ($p < 0.001$) and K_{ST}, the nucleotide sequence-based statistic estimated at 0.06995 ($p < 0.001$). Population comparisons conducted for the average number of pairwise nucleotide differences (K_{xy}) (Table 3) showed the lowest estimate of K_{xy} was 27.9 (Australia – New Zealand) and the highest was 44.3 (Central America – Southern California). For the nearest-neighbor statistic (S_{nn}; Table 3), values were lowest between Immature Hawaii and Japan (0.55) and highest between Australia and Mexico (0.95).

Pairwise genetic subdivision patterns were explored using the sequence-based statistic K_{ST} (Table 3). Because sequence-based methods utilize information not only based on the frequency of haplotypes, but also on the numbers of differences between haplotypes, they are powerful in detecting structure in longer sequences or within smaller sample sizes (Hudson et al. 1992; Hudson 2000). With the K_{ST} estimates, no significant differentiation was detected between any pairwise combination of the Japan, Mature Hawaii, Immature Hawaii, or Southern California locations. Also no population genetic structure was detected between Mexico and Central America or between New Zealand and Australia (Table 3). The K_{ST} estimates ranged from –0.0026 (Japan – Immature Hawaii) to 0.1172 (Australia – Central America).

A neighbor-joining haplotype tree (Fig. 3) was generated for the mtDNA control region sequences. For values over 50%, replicate trees where individuals clustered together in the bootstrap test (5000 replicates) are shown next to the branches. However, branches where separations were reproduced less than 50% of the time were collapsed. This tree showed some of the same spatial patterns revealed by the pairwise K_{ST} values, particularly clusters of individuals from Mexico, Central America, and Ecuador. However, other areas of the tree showed mixed regions of sequences from different locations, reflecting the overall low values of genetic differentiation.

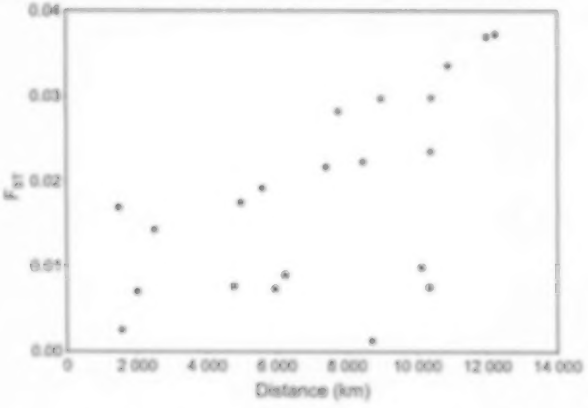
An AMOVA of spatial variation was computed by the distance matrix using pairwise distances in ARLEQUIN (10 000 permutations) for different groupings (Table 4), including several previous striped marlin stock models: northern vs. southern Pacific, western vs. eastern Pacific, and regional (Central America and Ecuador were grouped together because of the small sample size from Ecuador). Although, the majority of variation was contained within populations for all comparisons, those particular groupings did not minimize variation among populations within groups. The model that most minimized within-group variance, "Best fit" (Table 4), showed that although most variation (approximately 90%) occurred among samples within populations, about 10% (10.55%) of the variation was contained among groups, with effectively no variation among populations within groups. This model contained three groups: Mature Hawaii – Immature Hawaii – Japan – Southern California, New Zealand – Australia, and Mexico – Ecuador – Central America. Two alternate models were also provided for comparison. One was similar to the Best fit model except that Mature Hawaii was moved into a separate group, and while the AMOVA results were similar, slightly less variance was distributed among groups (9.77%). The second alternative moved Southern Cal-

Table 2. Weir and Cockerham's pairwise F_{ST} values (above diagonal) and Hedrick's G'_{ST} (below diagonal) for (*i*) null-corrected microsatellite data and (*ii*) original microsatellite data.

Population	JP	MT HW	IM HW	SC	MX	CA	NZ	AU
Null-corrected data								
JP		0.0074***	0.0035	0.0013	0.0099***	0.0076***	0.0224***	0.0284***
MT HW	0.0289		0.0066***	0.0077**	0.0193***	0.0176***	0.0090***	0.0219***
IM HW	0.0135	0.0263		0.0054	0.0154***	0.0131***	0.0121***	0.0173***
SC	0.005	0.0302	0.0196		0.0170***	0.0144***	0.0236***	0.0371***
MX	0.0337	0.0714	0.0539	0.0567		0.0026	0.0299***	0.0374***
CA	0.0273	0.0686	0.0483	0.0508	0.0087		0.0298***	0.0337***
NZ	0.0845	0.0358	0.0457	0.0862	0.1041	0.1103		0.0071
AU	0.1064	0.0879	0.0655	0.1345	0.1298	0.1233	0.027	
Original data								
JP		0.0081***	0.0002	0.0016	0.0070***	0.0077***	0.0216***	0.0263***
MT HW	0.0161		0.0029	0.003	0.0165***	0.0219***	0.0047	0.0183***
IM HW	0.0004	0.006		0.0003	0.0120***	0.0135***	0.0092***	0.0155***
SC	0.0031	0.0057	0.0008		0.0111***	0.0172***	0.0151***	0.0301***
MX	0.0135	0.032	0.0236	0.0206		0.0004	0.0308***	0.0377***
CA	0.0149	0.0432	0.0272	0.0332	0.0008		0.0340***	0.0335***
NZ	0.044	0.0094	0.0191	0.03	0.0615	0.0699		0.0083
AU	0.0549	0.0379	0.0327	0.0614	0.0771	0.0702	0.0177	

Note: Significant p values are denoted as follows: **, $p < 0.01$; ***, $p < 0.001$.

Fig. 2. Correlation between geographic distance (km) and population structure (F_{ST}) for striped marlin specimens ($R = 0.3559$), with significance determined by one-sided Mantel tests ($p = 0.0087$).



ifornia into the group with the rest of the eastern Pacific; however, results revealed this model was not nearly as good of a fit as the previous two groupings.

Discussion

Microsatellite analyses

Allelic richness and number of alleles were variable, depending on the locus and location. Heterozygosity levels were high but similar to what has been reported for striped marlin in a previous study (McDowell and Graves 2008) and in other pelagic fish such as Atlantic bluefin tuna (Carlsson et al. 2004) and Atlantic bigeye tuna (Gonzalez et al. 2008). Null alleles, a common cause of discrepancies between observed and expected heterozygosities, are known to be problematic and prevalent in microsatellite markers (Dakin and Avise 2004; Hedgecock et al. 2004; Kalinowski et al. 2006). In this study, null alleles were detected in a subset of loci and populations. While often mentioned as a caveat of population genetic data, usually no further treatment of the null alleles is attempted (Dakin and Avise 2004). However, given the detection of null alleles and the number of significant deviations in the heterozygosity and F_{IS} estimates, it was important to correct for null alleles in this data set, as it did change the number of significant pairwise relationships.

Overall F_{ST} and G'_{ST} values were low using microsatellites, but significant and in the range reported for other pelagic marine species (the reported median in Ward et al. 1994; O'Reilly et al. 2004; Rooker et al. 2007). Analysis of the eight groups of null-corrected samples revealed that striped marlin form four significantly different populations. The first group located in the southwest Pacific contained Australia and New Zealand. Australia had unusually high levels of genotypic disequilibrium, with 56% of locus-pairs showing significant disequilibrium, compared with only one other locus-pair (Mature Hawaii) in all other populations. One possible explanation was misidentification of other billfish species within that region's collection; however, all Australian samples had a striped marlin maternal background after sequencing the mitochondrial control region. Mexico and Central America in the eastern Pacific formed the second group; however, this group was significantly different from Southern California, despite also being in the eastern Pacific. The genetic heterogeneity found between Southern California and Mexico (primarily the Baja California region of Mexico) is surprising because of the close physical proximity between those two locations; yet it is also supported in the findings of McDowell and Graves (2008). Interesting spatial patterns were found in the northern Pacific. No significant differences were found between Japan and Southern California, but both were significantly different from Mature Hawaii. Interestingly, juvenile striped marlin caught around Hawaii were sig-

Table 3. Based on mitochondrial control region sequence analyses, (*i*) diversity estimates; (*ii*) K_{xy}; (*iii*) S_{nn}; and (*iv*) pairwise K_{ST} estimates.

Location	JP	MT HW	IM HW	SC	MX	CA	NZ	AU
Diversity estimates								
n_s	43	92	34	25	131	20	53	48
h	38	86	33	23	91	18	48	34
h_d	0.993	0.999	0.998	0.99	0.99	0.989	0.996	0.981
K	33	34	29	30	39	42	30	26
Pairwise K_{xy}								
MT HW	33.9							
IM HW	31.1	31.9						
SC	31.3	32.4	29.5					
MX	39.2	40.2	39.1	39.4				
CA	43.2	45.3	43.9	44.3	40.8			
NZ	33.2	33.5	30.6	32.2	39.9	44.1		
AU	31.7	32	28.8	30.9	38.9	43.5	27.9	
Pairwise S_{nn}								
MT HW	0.69**							
IM HW	0.55	0.62						
SC	0.57	0.69	0.61					
MX	0.92***	0.86***	0.89***	0.81***				
CA	0.85***	0.87***	0.83***	0.74**	0.79			
NZ	0.83***	0.69***	0.74**	0.83***	0.92***	0.81**		
AU	0.89***	0.77***	0.79***	0.89***	0.95***	0.94***	0.62*	
Pairwise K_{ST}								
MT HW	0.0025							
IM HW	–0.0026	0.0004						
SC	–0.0025	0.0048	–0.0006					
MX	0.0320***	0.0478***	0.0436***	0.0374***				
CA	0.0642***	0.0587***	0.1029***	0.1133***	0.0025			
NZ	0.0272**	0.0183***	0.0139*	0.0392***	0.0587***	0.0859***		
AU	0.0462***	0.0352***	0.0278**	0.0646***	0.0742***	0.1172***	–0.001	

Note: Diversity estimates include the number of sequences (n_s), number of haplotypes (h), haplotype diversity (h_d), and average number of differences between sequences (K); K_{xy} is the average number of pairwise nucleotide differences among sequences; S_{nn} is the nearest-neighbor statistic; and pairwise K_{ST} estimates are based on the sequence-based statistics of Hudson et al. (1992). Significant p values are denoted as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

nificantly different from mature fish in that area, but not significantly different from Japan and Southern California fish. Thus, according to the null-corrected data, striped marlin from Japan, California, and Immature Hawaiian fish form the third population, and Mature Hawaiian striped marlin constitute the fourth group.

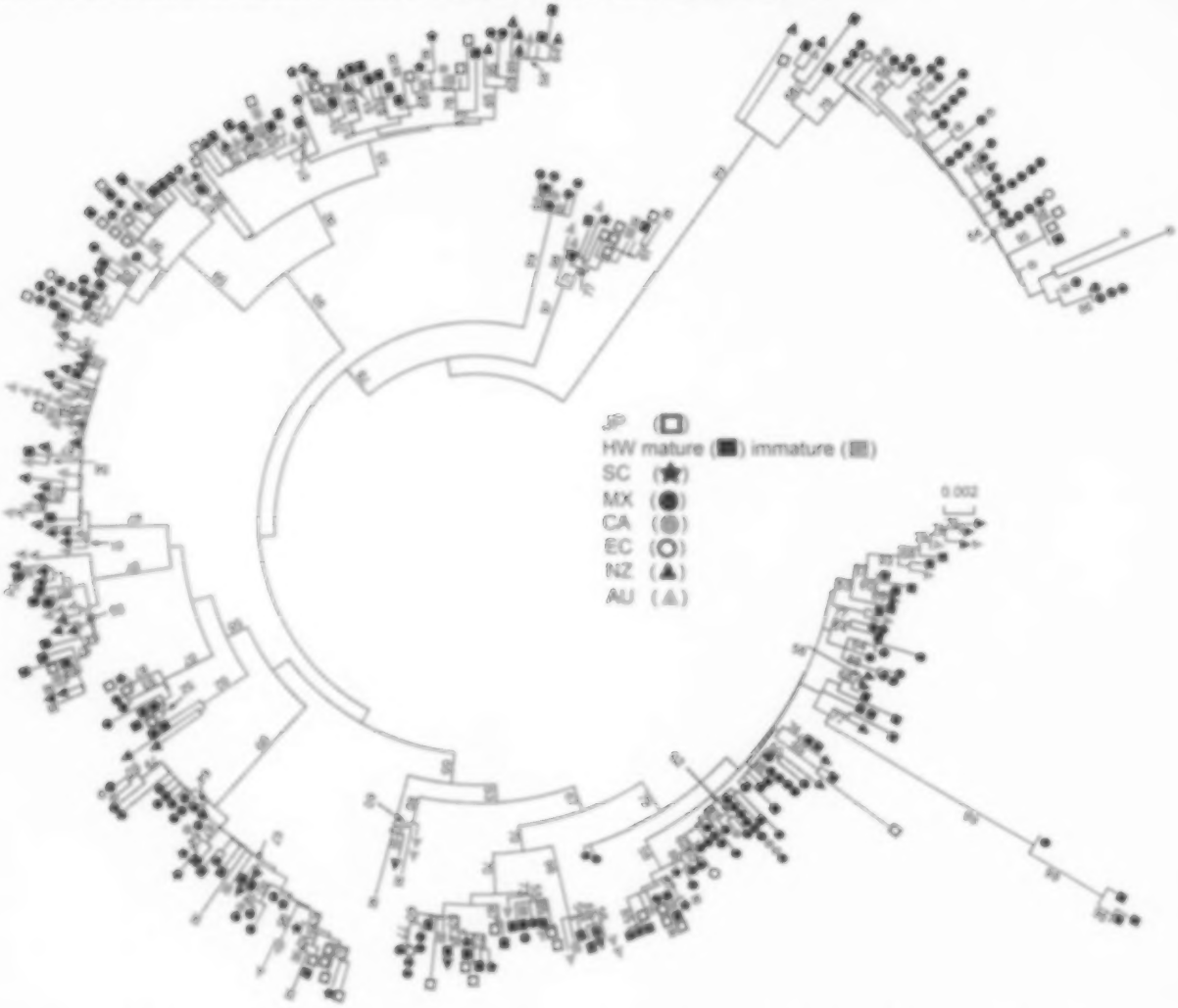
Despite the migratory behaviors that likely underlie the detected genetic patterns, there is still significant correlation between geographic distance and population structure, suggesting isolation by distance. Although some of these behaviors indicate trans-Pacific movement (Japan – Southern California), while others suggest limited mixing despite close proximity (Mexico – Southern California), in general, it appears that distance does limit genetic mixing, albeit at Pacific-wide scales. In contrast with pairwise analyses, the program STRUCTURE did not support more than one population of striped marlin in the Pacific. However, assignment methods have limited power when genetic differentiation is low among populations (Manel et al. 2005; Saenz-Agudelo et al. 2009).

When null allele frequencies were not incorporated into the data, overall F_{ST} and G'_{ST} estimates were lower, but many pairwise relationships remained the same. The primary difference was that Mature Hawaii showed fewer significant pairwise relationships; compared with null-corrected data no significant differences were detected with Southern California, Immature Hawaii, or New Zealand. For the original data, the pairwise F_{ST} values that changed in significance had some of the smallest significant values in the null-corrected data set. Thus, in this study, the null allele correction provided additional power to detect significant relationships rather than change the trends of those relationships. Interestingly, changes in significant pairwise groupings all involved Mature Hawaii, a group that contained the highest number of deviations in observed heterozygosity and F_{IS} estimates. This indicates that null allele correction was important to spatial heterogeneity analyses.

Mitochondrial DNA sequence analyses

Mitochondrial control region sequences contained a large number of haplotypes, and the haplotype diversity (h_d) was high but similar to other marine fish (Hauser et al. 2001;

Fig. 3. Neighbor-joining haplotype tree of mitochondrial control region sequences. Branches corresponding to separations reproduced less than 50% of the time are collapsed. The percentages of replicate trees where individuals clustered together in the bootstrap test (5000 replicates) are shown next to the branches for values over 50%. Symbols represent individual sequences.



Carlsson et al. 2004; Alvarado-Bremer et al. 2005). The eastern Pacific locations, Mexico and Central America, share the characteristics of having both the highest average numbers of differences between sequences (K), whereas Australia was lowest, which is perplexing given the unusual genotypic disequilibrium patterns in Australia mentioned above.

The overall genetic structure was significant using H_{ST} and K_{ST} estimates, and pairwise analyses showed patterns similar to the microsatellite analyses, with the same groups in the eastern Pacific (Mexico – Central America) and the southwest Pacific (Australia – New Zealand); however, there was a change in the North Pacific group. Sequence analyses did not reveal significant structure among Mature Hawaii and Japan, Immature Hawaii, or Southern California, and thus these specimens formed one group in the North Pacific. The nearest-neighbor statistic, S_{nn}, supported the K_{ST} results.

AMOVA comparisons also supported the mitochondrial pairwise results, with the best fit resulting in three groupings: (1) Australia and New Zealand, (2) Japan, Immature Hawaii, Mature Hawaii, and Southern California, and (3) Mexico, Central America, and Ecuador. Interestingly, the northern–southern and eastern–western stock model groupings proved to be a poor fit with the sequence data. The similarly poor fit of Southern California with the rest of the eastern Pacific sequences supports the pattern detected in the microsatellite analysis.

Overall spatial structure

Following the analysis of nuclear microsatellites and mitochondrial control region sequences, the spatial distribution and underlying migration patterns of striped marlin in the Pacific are starting to be understood. Large numbers of deviations in heterozygosity and F_{IS} values in mature Hawaiian specimens were at least partially explained by the presence of null alleles, but may also be indicative of a Wahlund effect,

Table 4. Analysis of molecular variance (AMOVA) of spatial variation in striped marlin control region sequences.

Structure tested	Variance	% Total	*F* statistics	*p*
West–east (MX, EC, CA, SC) vs. (JP, NZ, AU) vs. (MT HW and IM HW)				
Among groups	0.792 Va	4.23	F_{CT} = 0.0423	0.102
Among populations within groups	0.951 Vb	5.08	F_{SC} = 0.0530	<0.001
Within populations	16.988 Vc	90.69	F_{ST} = 0.0931	<0.001
North–south (EC, AU, NZ) vs. (JP, SC, MA HW, IM HW, MX, CA)				
Among groups	0.084 Va	0.45	F_{CT} = 0.0045	0.39
Among populations within groups	1.562 Vb	8.38	F_{SC} = 0.0842	<0.001
Within populations	16.988 Vc	91.16	F_{ST} = 0.0884	<0.001
Separate populations (IM HW and MT HW) vs. (CA, EC) vs. (JP) vs. (AU) vs. (SC) vs. (NZ) vs. (MX)				
Among groups	2.338 Va	12.57	F_{CT} = 0.1257	0.037
Among populations within groups	–0.730 Vb	–3.92	F_{SC} = –0.0449	<0.001
Within populations	16.988 Vc	91.35	F_{ST} = 0.0865	<0.001
All populations together (AU, MT HW, IM HW, JP, MX, NZ, SC, CA, EC)				
Among groups	1.605 Va	8.63	F_{CT} = NA	NA
Among populations within groups	NA	NA	F_{SC} = NA	NA
Within populations	16.988 Vb	91.37	F_{ST} = 0.0863	<0.001
Best fit (MT HW, IM HW, JP, SC) vs. (AU, NZ) vs. (MX, EC, CA)				
Among groups	2.001 Va	10.55	F_{CT} = 0.1055	<0.001
Among populations within groups	–0.013 Vb	–0.07	F_{SC} = –0.0008	<0.001
Within populations	16.988 Vc	89.52	F_{ST} = 0.1048	<0.001
Alternate group 1 (IM HW, JP, SC) vs. (MT HW) vs. (AU, NZ) vs. (MX, EC, CA)				
Among groups	1.831 Va	9.77	F_{CT} = 0.0977	<0.001
Among populations within groups	–0.085 Vb	–0.45	F_{SC} = –0.0050	<0.001
Within populations	16.989 Vc	90.68	F_{ST} = 0.0932	<0.001
Alternate group 2 (MT HW, IM HW, JP) vs. (MX, SC, EC, CA) vs. (AU, NZ)				
Among groups	1.266 Va	6.72	F_{CT} = 0.0672	0.003
Among populations within groups	0.576 Vb	3.06	F_{SC} = 0.0328	<0.001
Within populations	16.988 Vc	90.22	F_{ST} = 0.0979	<0.001

Note: Computed by the distance matrix using pairwise differences in Arlequin (10 000 permutations). AU, Australia; CA, Central America; EC, Ecuador; JP, Japan; IM HW, Immature Hawaii; MT HW, Mature Hawaii; MX, Mexico; NZ, New Zealand; SC, Southern California.

where sampling occurred across cohorts of juveniles or subgroups (Johnson and Black 1984; Lenfant and Planes 2002), especially as this location may serve as a stepping stone or feeding area for non-local striped marlin. There was disagreement between microsatellite and mitochondrial analyses; one possible reason may be lower resolution power with the fewer analyzed mtDNA sequences compared with the number of specimens analyzed with microsatellites. Alternatively, microsatellites may be more sensitive than mtDNA sequences, reflecting very low levels of gene flow between locations (Feulner et al. 2004; Keeney et al. 2005). It is also possible that Japanese juveniles migrating to (or through) Hawaii may be reflected in samples collected in that location, and mixing of these groups could impact analyses for both mature and immature Hawaiian samples. Japanese juveniles around Hawaii may grow and be included in analyses of mature Hawaiian fish while still not reproductively contributing to this location. Similarly, although spawning in striped marlin is now confirmed in Hawaii (Hyde et al. 2006), collections of Hawaiian juveniles likely have Japanese fish mixed in. Movement of juvenile striped marlin into Hawaiian waters is supported by previous reports (Squire and Suzuki 1990), where the fish likely use the area as a feeding ground before moving on to spawning locations (Matsumoto and Kazama 1974).

Overall, the North Pacific shows very little spatial subdivision among the Japan, Immature Hawaii, Mature Hawaii, and Southern California locations. The seasonal Southern California location does not have a known spawning location, and striped marlin in this area likely originate in Japan (Nishikawa et al. 1978). Fish from Japan may move eastward with the Kuroshiro Current to feeding grounds near the Hawaiian Islands; when currents or environmental conditions are right, some fish may continue eastward until they reach Southern California. The seasonal population in Southern California is not large, but their movement into the region is a regular occurrence, although the timing and number of fish each year varies. While it is uncertain how long the fish remain in this region, they may eventually utilize the North Equatorial Current to move back across the Pacific to their spawning grounds in Japan, or they may represent a population sink, not reproductively contributing to any population.

Based on the genetic analyses, Mexican and Central Amer-

ican striped marlin appear to form one stock that is differentiated from other Pacific populations. Despite the genetic exclusion of Southern Californian striped marlin from the rest of the eastern Pacific, tagging data indicate that striped marlin caught in Southern California move south into Baja California, Mexico, corresponding to cooling water temperatures off California (Domeier 2006). However, the Southern California fish do not appear in representative samples from Mexico; this may happen for a couple of reasons. First, their behavior in Mexico may reduce their catchability to the recreational fishery, which was the primary sample source in that region. This is plausible because the spawning season for the Mexican population coincides with the southward movement of Southern California fish, and evidence suggests that local striped marlin shift locations in preparation for spawning (Armas et al. 1999; Domeier 2006). Tournament catch rates indicate that the Mexican striped marlin population is far larger than the Southern California population. It would seem logical for recreational and commercial fishers to shift their effort to follow the larger, year-round population of Mexican fish to their spawning grounds. As the striped marlin from Southern California do not appear to be spawning in Mexico, they may largely escape the fishing pressure by not moving to the spawning locations. Alternatively, the Southern Californian striped marlin may not stay long in the waters off of Mexico, but rather move through Mexico to follow currents back across the Pacific. Through these (and possibly other) behavioral modifications, and with the relative size of the Southern California population compared with the Mexico population, it is not surprising that they are not significantly sampled within this region.

In the southwest Pacific, Australia and New Zealand form another independent stock. Australia displayed unusual genetic variability patterns, with high numbers of locus-pairs in genotypic disequilibrium and heterozygosity deviations. One possibility is gene flow occurring with Indian Ocean striped marlin on a semiregular basis, given the right environmental conditions (Bromhead et al. 2004), but with little genetic work done on Indian Ocean striped marlin, it is difficult to determine if mixing is driving these differences. If Pacific–Indian Ocean mixing was occurring, sequence variation would be expected to be higher here than in other locations, and the results do not support this hypothesis. However, if gene flow from the Indian Ocean was primarily male-mediated, then mitochondrial sequence variation would not be greatly impacted. Hybridization with other regional billfish species is another possibility. All maternal backgrounds were striped marlin, but a unidirectional hybrid cross is possible, though this has not been reported in billfish previously. Like Southern California, striped marlin in New Zealand are seasonal. The New Zealand fish likely originate from Australia and perhaps from other unsampled areas in the Central–South Pacific (Bromhead et al. 2004); they are noted for relatively high dispersal distances, likely correlated with changing water temperatures, in contrast with Australian striped marlin that make much shorter distance coastal shifts (Kopf et al. 2005; Domeier 2006).

The presumed large-scale movements underlying the genetic subdivision patterns of striped marlin during different portions of their life history, particularly in the North Pacific, demonstrate the need for a concurrent, temporally, and spatially representative sampling approach. While results of this study were similar to McDowell and Graves (2008), they did not analyze immature and mature fish separately; therefore no structure was detected between those groups in Hawaii. McDowell and Graves (2008) also found structure between Mexico and Ecuador, while in this study Mexico and Central America comprised a single stock. This difference is difficult to explain, as the larger numbers of markers and samples used in this study should increase resolution. One possible explanation is that the more southern location, Ecuador, showed stronger differentiation than Central America in this study; however, the collecting range for specimens from these two locations were relatively close geographically. Alternatively, this may represent sampling artifact in McDowell and Graves (2008), caused by relatively small, single collections in those locations (Mexico: $n = 32$, 1994; Ecuador: $n = 39$, 1995). Despite these differences, overall values of F_{ST} were similar (using microsatellites) between this study and the McDowell and Graves 2008 study (0.0145 and 0.0130, respectively). This study also showed concordance between the two molecular markers, including the same significant pairwise relationships (with the exception of mature Hawaiian specimens). This suggests that small sample size was likely the reason that McDowell and Graves (2008) did not detect significant subdivision in pairwise mtDNA estimates.

Conservation implications

While it is not easy to subdivide migratory species into distinctly manageable units, there are patterns from this study that can be applied at a management level. Based on all specimens, the north Pacific (Japan, Mature Hawaii, Immature Hawaii, and Southern California) showed little genetic differentiation among locations. However, subtle population structure between mature Hawaiian specimens and the other locations in this group indicates that effective migration (migration with reproductive contributions) between Hawaii and other North Pacific locations is probably limited. Therefore, it may be more accurate to consider them as separate stocks even though fishing efforts in Hawaii can impact both Hawaiian and Japanese striped marlin populations. The Hawaiian location is clearly important to several populations within the North Pacific, and because of this connection, temporal analyses may be very informative in managing this area. Next, Mexico and Central America appear differentiated from the other Pacific locations. Despite the movement of Southern Californian fish through that region, the eastern Pacific should be managed as a separate stock. Finally, the Southwest Pacific (Australia and New Zealand) form another distinctive stock. Australia, in particular, may be impacted by migration or hybridization in ways that are different from other regions in the Pacific.

Based on the analysis of over 1000 specimens collected from seven locations, using two classes of molecular markers, striped marlin populations showed shallow but significant overall genetic subdivision with evidence for at least three (Australia – New Zealand, Japan – Southern California – Hawaii, Mexico – Central America) and possibly four (Mature Hawaii) putative stocks in the Pacific. What may be more complicated is the level of independence of these groups, particularly with the connections in the North Pacific and possibly the Australian – New Zealand group with poten-

tial ties to the Indian Ocean. However, for a pelagic species capable of long migrations, striped marlin show considerable spatial variation throughout their range in the Pacific.

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Introgressive hybridization among major Columbia River Chinook salmon (*Oncorhynchus tshawytscha*) lineages within the Klickitat River due to hatchery practices

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Abstract: Major lineages of anadromous salmonids show resilience to natural introgressive hybridization; however, Klickitat River spring-run Chinook salmon (KRSC, *Oncorhynchus tshawytscha*) have an enigmatic origin because of their intermediate genetic and geographic relationship among Columbia River Chinook salmon lineages. We used computer simulations to evaluate four anthropogenic and natural processes as likely causes of the apparent introgressed genetic composition of KRSC: recent admixture (~5 generations), historical admixture (>200 generations), isolation-by-distance gene flow, and natural selection. We also genotyped 2413 fish (32 collections) across 96 single nucleotide polymorphism loci to clarify the relationship of KRSC among the three major Columbia River lineages (Lower Columbia and interior ocean- and stream-types) and to quantify introgression among collections. Between 1980 and 2000, we observed a decline of pure interior stream-type individuals in the KRSC collections. This temporal shift in genetic composition was coincident with relevant changes in hatchery practices. Based on results from the simulations and time-series samples, a recent and anthropogenically caused admixture was most likely responsible for introgression of KRSC. Potential long-term negative effects of introgression may require some form of mitigation.

Résumé : Les lignées principales de salmonidés anadromes montrent de la résilience à l'hybridation naturelle par introgression; cependant, les saumons chinook de la montaison printanière de la rivière Klickitat (KRSC, *Oncorhynchus tshawytscha*) ont une origine énigmatique, à cause de leur relation génétique et géographique intermédiaire parmi les lignées de saumons chinook du fleuve Columbia. Des simulations à l'ordinateur nous ont servi à évaluer quatre mécanismes anthropiques et naturels comme causes vraisemblables de la composition récente des KRSC avec introgression apparente soit, un mélange récent (~5 générations), un mélange ancien (>200 générations), un flux génique avec isolement en fonction de la distance et la sélection naturelle. Nous avons aussi déterminé le génotype de 2413 poissons (32 récoltes) à l'analyse de 96 locus de polymorphisme mononucléotidique afin de clarifier les relations des KRSC avec les trois autres lignées principales du Columbia (Columbia inférieur, types océanique et riverain de l'intérieur) et de mesurer l'introgression entre les récoltes. Entre 1980 et 2000, nous observons un déclin des individus de type riverain pur de l'intérieur dans les récoltes de KRSC. Ce changement temporel dans la composition génétique coïncide avec des modifications pertinentes dans les pratiques de pisciculture. D'après les résultats des simulations et des séries chronologiques d'échantillons, le plus vraisemblable est qu'un mélange récent d'origine anthropique soit responsable de l'introgression des KRSC. Les effets négatifs potentiels à long terme de cette introgression pourraient nécessiter des formes de mitigation.

[Traduit par la Rédaction]

Introduction

Introgressive hybridization occurs when two distinct species or populations produce hybrid offspring that subsequently backcross with parental types. Both natural and anthropogenic processes can lead to introgressive hybridization. Natural hybridization may be beneficial in terms of gene flow and diversity, but when it occurs via anthropogenic influences, hybridization can have negative impacts on fitness of the introgressed population (e.g., Rhymer and Simberloff 1996; Gilk et al. 2004). At the interspecific level, necessary protective measures are available to deal with the causes and outcomes of hybridization. Although detecting hybridization among conspecifics may be more challenging,

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it is critical to address intraspecific hybridization for population management and requires the ability to discriminate natural from human-mediated causes (Allendorf et al. 2001).

The natural distribution of Chinook salmon (*Oncorhynchus tshawytscha*) spans the Pacific Rim. Diverse life history traits occur across the range (Waples et al. 2004), making this an ideal species for examining intraspecific hybridization. The Columbia River Basin is a central location of genetic diversity, abundance, and distribution for Chinook salmon. It consists of 670 000 km^2 in the northwestern USA and Canada and once supported an estimated four million Chinook salmon (Chapman 1986). Despite recent population declines in the basin, genetically distinct Chinook salmon lineages still persist and represent divergent suites of life history traits (Brannon et al. 2004). Further, some populations have warranted federal protection under the Endangered Species Act to improve declining abundances and conserve their unique suites of adaptations (Good et al. 2005).

The potential negative fitness impact of intraspecific introgression (i.e. outbreeding depression) may be difficult to predict (McClelland and Naish 2007). However, anadromous salmonid populations that are highly diverged and also distinguished by life history traits may be especially sensitive to disruption of co-adapted gene complexes that can occur in F_2 and later generation hybrids (e.g., Gharrett et al. 1999). Nevertheless, major lineages of anadromous salmonids appear to be largely resilient to natural introgressive hybridization, likely because of fitness advantages they gain within their specific ecological niches (Utter 2000; Narum et al. 2007). Even when natural reproductive barriers are present, it is still possible to induce introgression through either indirect or direct human-mediated action (e.g., Bartley et al. 1990; Spies et al. 2007). In addition, the dynamic geologic history of the Columbia River Basin may have provided a rare set of conditions that have led to populations in which introgressive hybridization has occurred naturally (Waples et al. 2008).

Chinook salmon in the Columbia River Basin consist of three main genetic lineages (Waples et al. 2004; Narum et al. 2010), referred to as the Lower Columbia, interior ocean-type, and interior stream-type lineages. The interior ocean- and stream-type lineages are sympatric and correspond with divergent life history strategies including differences in juvenile migration timing, adult spawning location, marine distribution, and run timing (Healey 1991; Waples et al. 2004). Adult run timing of interior ocean-type populations is later in the summer and fall compared with the earlier run timing of interior stream-type populations in the spring and summer. The Lower Columbia lineage is composed of both spring- and fall-run populations, but they are not similarly differentiated by the distinct life history attributes that distinguish the interior lineages (Waples et al. 2004). Phylogenetic analyses of these lineages using both allozymes (Utter et al. 1989; Marshall et al. 1995) and microsatellites (Narum et al. 2010) indicate relative genetic similarity between the Lower Columbia and interior ocean-type lineages compared with greater genetic distance between each and the interior stream-type lineage. Further, those results consistently identify a population of Klickitat River spring-run Chinook (KRSC) salmon with an intermediate phylogenetic relationship between the interior stream-type lineage and the Lower Columbia – interior ocean-type lineages.

In this evaluation, we examined three natural processes that may explain the intermediate phylogenetic nature of the KRSC population: historical admixture, isolation-by-distance gene flow, and natural selection. Historical admixture of lineages may have occurred during recolonization of the landscape following glacial isolation (Waples et al. 2008). Following deglaciation, expanding ranges for Lower Columbia and interior stream-type lineages may have converged at a natural contact zone near Celilo Falls (river kilometre (rkm) 320 of the Columbia River), in the proximity of the Cascade Mountain crest. Contact would have occurred approximately 12 000 – 10 000 years ago (Gradstein et al. 2004). A similar genetic signature associated with this contact zone has been documented in other Columbia River salmonids (Utter and Allendorf 1977; Taylor et al. 1999). Alternatively, given that the Klickitat River subbasin is geographically equidistant from the centers of distribution of the major genetic lineages, it is possible that isolation-by-distance gene flow is responsible for the observed distinction of KRSC among lineages. Finally, natural selection may produce similar genetic patterns as those that arise from hybridization (e.g., linkage disequilibrium across large genomic intervals; Kohn et al. 2000). Natural selection can also diverge populations from each other if different selection regimes are operating within them and therefore is also a potential force that could be acting to diverge the KRSC population.

Whether or not natural processes have contributed, anthropogenic activities may have had the greatest genetic impact on the native KRSC. Prior to habitat alterations and other landscape modifications stemming from hatchery practices, natural cataracts in the lower Klickitat River restricted access to most of the river. Only spring-run Chinook salmon that returned during high flows could navigate the falls (Myers et al. 2006). The origins of those historical KRSC are unknown and may have been derived from either the Lower Columbia or interior stream-type lineages. In 1946, construction of fish ladders and modifications at natural waterfalls allowed protracted upstream passage of fish. A fall-run Klickitat Hatchery program began that year, and initial broodstocks were derived from Lower Columbia stocks. Later in 1986, a switch was made to an interior ocean-type summer–fall-run broodstock (Marshall et al. 1995). The Klickitat Hatchery also began a spring-run Chinook salmon program in 1952, utilizing fish collected at a trap on the Klickitat River (Marshall et al. 1995). Between 1950 and 1990, out-of-basin stocks of spring- and fall-run fish were released to augment hatchery production (Table 1). Integration of exogenous stocks into Klickitat hatchery broodstocks could have resulted in a recent admixture.

Our study employed a combination of empirical and simulated data to determine the most likely of four natural and anthropogenic scenarios that may have led to the contemporary genetic composition of KRSC. We genotyped 32 collections of Columbia River Chinook salmon, including KRSC collections and reference populations from the three lineages, using a panel of 96 single nucleotide polymorphism (SNP) loci. The KRSC collections span 28 years (1980–2008) and include marked hatchery fish, unmarked natural-origin fish, and hatchery broodstock fish. We confirmed the phylogenetic status of KRSC and looked for evidence of a recent tempo-

Table 1. Major events in out-of-basin Chinook salmon stocking history of the Klickitat River subbasin.

Year	Impact years	KRSC impact	Lineage	Marine distribution	Spawning habitat	Smolt age	Morphology	Migration timing
1946–1986	1951–1995	a	Lower Columbia	BC, WA	Mainstem	0+	D	Fall
1977–1979	**1982–1988**	b	Interior ocean-type	AK, BC	Mainstem	0+	B	Su–Fall
1987, 1991	**1992–2000**	c	Interior ocean-type	AK, BC	Mainstem	0+	B	Su–Fall
1978, 1980	**1983–1989**	d	Interior ocean-type	AK, BC	Mainstem	0+	B	Fall
1986–present	**1992–present**	e	Interior ocean-type	AK, BC	Mainstem	0+	B	Fall
1964–1984	1969–1993	f	Lower Columbia	AK*, BC, WA	Headwater	0+, 1+*	B	Sp
1961–1987	1966–1996	g	Interior stream-type	Offshore	Headwater	1+	B	Sp–Su

Note: Impact years indicates the approximate time frame required for released fish (KRSC impact) to return as adults (including jacks) and produce F_1 progeny that return as 3-, 4-, and 5-year-olds. Bold year entries indicate a shift in hatchery practice coincident with a KRSC genetic change after 1984. KRSC impacts: (a) broodstock origin: Spring Creek – Cowlitz River Hatchery, (b) exogenous releases (<50 000 per year): Wells Hatchery yearlings, (c) exogenous releases (500 000 – 1 200 000): Wells Hatchery yearlings, (d) exogenous releases (2 500 000 – 6 000 000): Bonneville Hatchery yearlings, (e) broodstock origin: switched to Priest Rapids Hatchery stock, (f) exogenous releases (200 000 – 1 000 000): Cowlitz River, Bitter Creek, Willamette River, (g) exogenous releases (6 000 – 600 000): Carson Hatchery. Biological information for Chinook salmon stocks was gathered from Marshall et al. (1995) and Myers et al. (1998). Hatchery impacts were compiled from Klickitat Hatchery records. The marine coastal distributions are British Columbia, Canada (BC), Washington State (WA), and Alaska (AK). Morphology characterizes returning adults as either with dark (D) or bright (B) coloration at freshwater entry. Smolt age is denoted as subyearling (0+) or yearling (1+). Seasonal migration timing is either fall-run (Fall), spring-run (Sp), or summer-run (Su).

*Some fish attributes are primarily characteristic of Willamette River spring-run Chinook salmon.

Table 2. Hypotheses evaluated to explain KRSC intermediate genetic characterization among Columbia River Chinook salmon lineages.

Causal force	Process	KRSC description	Generations back	Migration rate	Population expectations
Natural	Historical admixture (sudden)	Lineage contact	200	0.9	Intermediate (+); no shift
	Historical admixture (gradual)	Lineage contact	200	0.009	Intermediate (+); no shift
	IBD gene flow	Stepping stone model	2000	0.009	Intermediate (–)*
	Selection	Selection favoring intermediate allele frequency	2000	0.01	Intermediate (–); high LD
Anthropogenic	Recent admixture (sudden)	Hatchery introgression	5	0.9	Intermediate (+); shift
	Recent admixture (gradual)	Hatchery introgression	5	0.009	Intermediate (+); shift

Note: The term "intermediate" refers to a phylogenetic distinction of the Klickitat River spring-run population involving introgression (+) or no introgression (–). An asterisk (*) indicates significant (i.e., $P < 0.05$) regression of geographic distance on genetic distance, or isolation-by-distance (IBD). LD refers to linkage disequilibrium of large sections of the genome caused by positive selection on a small number of loci. "Shift" refers to change in genetic composition of KRSC collections between the 1980s and early 2000s.

rally based change in genetic composition of KRSC coincident with changes in the hatchery program. The large number of loci and high genetic divergence among major lineages helped overcome some technical challenge, and we characterized and quantified the levels of introgression using standard hybrid detection analyses. Patterns of introgression resulting from the simulated natural and anthropogenic processes were compared with those patterns observed in the KRSC collections to determine which of four described processes best fit our empirical results (Table 2).

Materials and methods

Data collection

Individuals ($n = 2413$) from 19 collections were chosen as a baseline to represent the three major genetic lineages of the Columbia River Basin, an additional twelve collections were used to represent KRSC, and one collection was included to represent the Klickitat River summer-run Chinook salmon (Table 3; Fig. 1). Most collections were sampled within the past two decades (1993–2008), but the KRSC collections included earlier collections from the 1980s. The KRSC collections were chosen to include hatchery as well as natural-origin fish and included fish selected for hatchery broodstock in years 2006–2008. The 19 baseline collections were selected to obtain balanced representation of the geographic distribution of each genetic lineage, and to provide an accurate portrayal of genetic diversity of native stocks. Where possible we used natural-origin collections and avoided hatchery populations derived from out-of-basin broodstock. It should be noted that the Spring Creek National Fish Hatchery collection was used as a proxy for the population of the fall-run Chinook salmon that historically inhabited the Big White Salmon River prior to dam construction in 1913, which excluded salmon from upstream migrations (Spring Creek in Fig. 1); the program was initially developed using eggs of natural-origin adult spawners from the Big White Salmon River (Marshall et al. 1995).

Tissue samples from each individual were processed with Qiagen DNeasy kits to extract DNA. For most collections, the tissue consisted of fin clips stored in 100% ethanol; however, four collections (Klickitat_spH_80, Klickitat_spH_84, Klickitat_spH_85, and Klickitat_su_85) came from archived scale cards that were stored dry. Details on the panel of 96 SNP loci and the protocols for genotyping these loci are described in Matala et al. (2011).

Table 3. Chinook salmon collection descriptions.

							Assignment			Descriptive statistic		
Collection ID	*n*	Lineage	Origin	Run	Range	Year	LC	OT	ST	*P*	F_{IS}	LD
1. McKenzie	84	LC	Hat	Sp	NA	2004	**0.93**	0.04	0.02	0.6598	–0.006	1
2. Cowlitz	86	LC	Hat	Fa	NA	2004	**0.92**	0.06	0.02	0.4963	0.000	3
3. Kalama	81	LC	Hat	Sp	NA	2004	0.76	0.07	0.17	0.0010	0.041	18
4. Lewis	93	LC	NOR	Fall	NA	2003	**0.87**	0.09	0.04	0.0493	0.021	1
5. Spring Creek	88	LC	Hat	Fall	NA	2006	**0.96**	0.04	0.00	0.7695	–0.011	1
6. Klickitat_fa_99	45	OT	NOR	Fall	10/07 – 10/08	1999	0.05	**0.94**	0.01	0.1125	0.023	0
7. Klickitat_fa_04	47	OT	NOR	Fall	Unknown	2004	0.06	**0.93**	0.01	0.0626	0.029	0
8. Lower Deschutes	90	OT	NOR	Fall	NA	1999	0.10	**0.89**	0.01	0.0071	0.033	0
9. Hanford	93	OT	NOR	Su–Fall	NA	2000	0.04	**0.95**	0.01	0.3162	0.006	0
10. Wells Dam	89	OT	Hat	Su–Fall	NA	1993	0.04	**0.94**	0.03	0.0171	0.029	1
11. Lyons Ferry	90	OT	Hat	Fall	NA	2002	0.07	**0.92**	0.01	0.0527	0.022	0
12. Clearwater	213	OT	NOR	Fall	NA	2008	0.07	**0.92**	0.01	0.9999	–0.032	2
13. John Day	84	ST	NOR	Sp	NA	2005	0.02	0.02	**0.95**	0.1132	0.017	2
14. Cle Elum	88	ST	Hat	Sp	NA	2006	0.05	0.07	**0.88**	0.0297	0.025	3
15. Wenatchee	85	ST	NOR	Sp	NA	1993	0.01	0.01	**0.99**	0.1201	0.017	2
16. Methow	93	ST	Hat	Sp	NA	1998	0.01	0.02	**0.97**	0.0002	0.048	2
17. Imnaha	92	ST	NOR	Sp–Su	NA	1998	0.01	0.01	**0.98**	0.7024	–0.008	2
18. Lolo	89	ST	NOR	Sp–Su	NA	2001	0.01	0.01	**0.98**	0.5053	0.000	2
19. Big Creek	92	ST	NOR	Sp–Su	NA	2001	0.01	0.01	**0.99**	0.4042	0.003	4
Klickitat (KRSC)												
20. spH_80	21	Test	U	Sp	5/10 – 5/24	1980	0.09	0.05	**0.87**	0.0005	0.092	0
21. spH_84	60	Test	U	Sp	5/11 – 6/10	1984	0.24	0.04	0.72	**<0.0001**	0.133*	72
22. spH_85	54	Test	U	Sp	5/01 – 6/30	1985	0.18	0.09	0.73	**<0.0001**	0.068*	18
23. su_85	83	Test	U	Su	6/01 – 10/31	1985	0.47	0.15	0.38	**<0.0001**	0.185*	178
24. spH_02	47	Test	Hat	Sp	Unknown	2002	0.11	0.07	**0.82**	0.4498	0.002	2
25. spH_06	85	Test	Hat	Sp	5/12 – 7/5	2006	0.14	0.10	0.76	0.1820	0.012	3
26. spBRST_06	38	Test	Hat	Sp	Unknown	2006	0.18	0.08	0.74	0.0590	0.030	2
27. spNOR_06	50	Test	NOR	Sp	5/12 – 7/25	2006	0.24	0.14	0.62	0.0002	0.059	4
28. spH_07_08	25	Test	Hat	Sp	5/09 – 7/18	2007–2008	0.12	0.08	0.80	0.0327	0.045	1
29. spBRST_07	46	Test	Hat	Sp	Unknown	2007	0.18	0.10	0.72	0.0109	0.040	3
30. spNOR_07	77	Test	NOR	Sp	4/16 – 7/12	2007	0.19	0.10	0.71	0.0001	0.050	3
31. spBRST_08	45	Test	Hat	Sp	Unknown	2008	0.22	0.07	0.70	0.0019	0.052	2
32. spNOR_08	60	Test	NOR	Sp	5/05 – 7/02	2008	0.23	0.10	0.68	0.0830	0.021	3

Note: Total sample size (*n*) is 2413. Putative lineages of origin are Lower Columbia (LC), interior ocean-type (OT), and interior stream-type (ST). Origin is natural (NOR), hatchery (Hat), or unknown (U). Under the Run column, Sp is spring-run and Su is summer-run. Range indicates the inclusive sample dates (month/day) for Klickitat River collections; all other collections are not applicable (NA). Abbreviations for Klickitat River collection nomenclature include sp (spring-run), su (summer-run), fa (fall-run), H (hatchery), BRST (broodstock), NOR (natural origin), and numbers for calendar year during which collections were obtained. Assignments to lineage are the membership coefficient of STRUCTURE for $k = 3$ (bolding indicates >80% membership). Under descriptive statistics, *P* is the probability result of Hardy–Weinberg equilibrium (HWE) tests, where F_{IS} indicates heterozygote deficiencies (positive values); significant results are indicated with bold font and also with an asterisk (*). The numbers of locus-pairs indicating significant linkage disequilibrium (LD) are provided for all 32 collections in the study.

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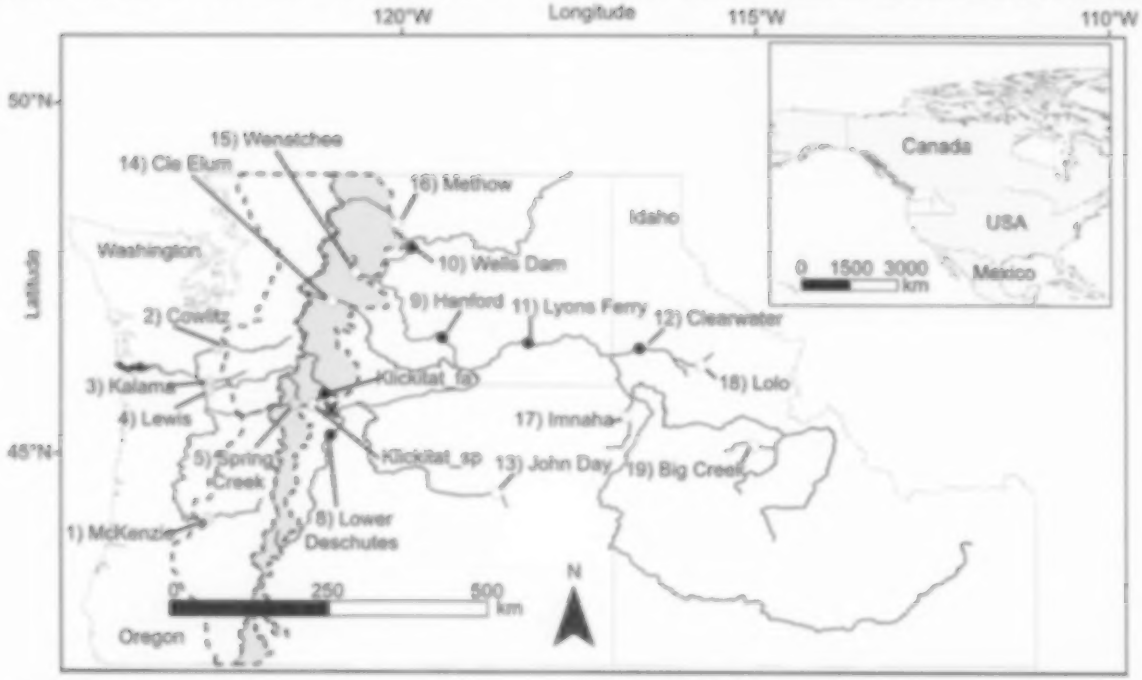
Fig. 1. Distribution of Chinook salmon collections within the Columbia River Basin. The grey, black, and white circles indicate collections of the Lower Columbia, interior ocean-type, and interior stream-type lineages of Chinook salmon, respectively. An X indicates the historical location of Celilo Falls, which was once a major barrier to salmon dispersal before being inundated by the Dalles Dam. Dashed outlines delineate the western and eastern sides of the Cascade Mountain Range in white and grey shading, respectively. Corresponding numbered collections are described in Table 3. "Klickitat_fa" represents the fall-run collections (6–7), and "Klickitat_sp" represents the spring-run collections (20–32) from the Klickitat River. The inset map shows the area of the Columbia River Basin (in white) in relation to North America.



Statistical analyses

We initially genotyped each individual at a total of 96 SNP loci. However, two loci were found to be monomorphic in the Columbia River Basin and were excluded from all analyses. The remaining 94 loci were tested for deviations from Hardy–Weinberg equilibrium (HWE) and were tested in pairs for linkage disequilibrium using GENEPOP v. 4.0.10 (Raymond and Rousset 1995); run settings were 1000 dememorizations, 100 batches, and 1000 iterations per batch. F_{IS} and pairwise F_{ST} values were estimated with FSTAT v. 2.9.3.2 (Goudet 2002). Stream distances were calculated based on data from the StreamNet project using the STREAM DISTANCE application (D. Graves; available from http://maps.critfc.org/file_download/StreamDistanceApplication.zip). Isolation-by-distance gene flow was examined using a Mantel test of pairwise F_{ST} and pairwise stream distance with 9999 permutations performed in GenAlEx v. 6.3 (Peakall and Smouse 2006).

A pairwise genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distances was generated using the software program PHYLIP v. 3.5C (Felsenstein 1989). The NEIGHBOR, SEQBOOT, and CONSENSE applications in PHYLIP were used to construct an unrooted neighbor-joining (NJ) consensus tree with bootstrap support. The matrix of chord distance values was used to construct an NJ dendrogram to show scaled genetic distances among collections, and this tree was drawn with the program FIGTREE v. 1.1.1 (Rambaut 2006). The PHYLIP package is available from the following link: http://evolution.genetics.washington.edu/phylip/.

STRUCTURE v. 2.3.2 (Pritchard et al. 2000) was used to determine individual assignment to three clusters, corresponding to the three major genetic lineages represented in our data set. An initial burn-in of 20 000 iterations followed by 200 000 iterations of Markov chain Monte Carlo (MCMC) method was used to generate posterior probabilities for each of 40 trials using setting $K = 3$ (corresponding to the three lineages). To obtain a set of robust individual assignment, a membership coefficient (Q) was calculated for each of $K = 3$ clusters, and the FullSearch algorithm in CLUMPP (Jakobsson and Rosenberg 2007) was used to find the optimal alignment of clusters across multiple runs. Only the top ten replicate STRUCTURE trials (R) with highest mean ln[Pr(X/K)] were averaged using the FullSearch algorithm in CLUMPP. The total number of all possible alignment configurations was computed by the equation $(K!)^{R-1}$, which results in a total of 10 077 696 possible configurations that were examined for the highest pairwise similarity (H') to achieve optimal alignment. The locus *Ots_C3N3* is a mitochondrial SNP and was coded as haplotypic data in STRUCTURE by listing only one allele and indicating the second allele as missing data.

NEWHYBRIDS v. 1.1beta (Anderson and Thompson 2002) was used to assign all individuals from across collections to the following six default genotype classes: pure_0 parental, pure_1 parental, F_1, F_2, 0_backcross, and 1_backcross. Since the program accepts only two parental types, we grouped the Lower Columbia and interior ocean-type collections into the pure_0 parental category based on similarity of

the lineages (Narum et al. 2010), and the interior stream-type collections were placed into the pure_I parental category (Table 3). We used the following parameter settings: an initial 50 000 iteration burn-in, followed by 500 000 iterations MCMC, and Jeffrey's prior for π and for θ. The KRSC collections were treated as the "test" category for assigning membership to one of the six genotype classes. The mitochondrial locus *Ots_C3N3* was omitted for NEWHYBRIDS analyses.

COLONY (Wang 2004) was used to determine whether KRSC collections contained full-sibling families. We ran each collection separately using a medium length run without prior information on candidate parents. 90 SNP loci were analyzed with a 0.003 error rate to account for the average genotyping error estimated for these loci used in our laboratory. Potential effects of full-sibship were examined by including only one individual from each putative full-sibling family and re-analyzing estimates of introgression in KRSC. Putative full-siblings were determined as those individuals with greater than 99% probability of full-sibship.

Simulations

The program HYBRIDLAB v .1.0 (Nielsen et al. 2006) was used to simulate multilocus backcross genotypes from real emperical data that was input from our Chinook salmon collections. We first used a random sample (n = 100) from the combined Lower Columbia and interior ocean-type collections and a random sample (n = 100) from the interior stream-type collections to create two parental populations. The F_1 genotypes (n = 100) generated from crossing parental types was then treated as a parent collection and crossed with the interior stream-type parental mixture to obtain backcross genotypes (n = 100). In additional simulations to test the accuracy of the method, we specified the Klickitat River fall-run interior ocean-type collection (n = 92) and the interior stream-type collection from the Wenatchee River (n = 85) as parental populations. These collections were chosen because they were geographically the closest "pure" collections in our study that represented interior ocean- and stream-type lineages. A file generated of F_1 genotypes (n = 85) was then used as a parent collection and crossed with the Wenatchee River collection to obtain backcross genotypes (n = 85). The Klickitat River interior ocean-type and the Wenatchee River interior stream-type collections were then analyzed along with the HYBRIDLAB backcrosses in the program NEWHYBRIDS as described above.

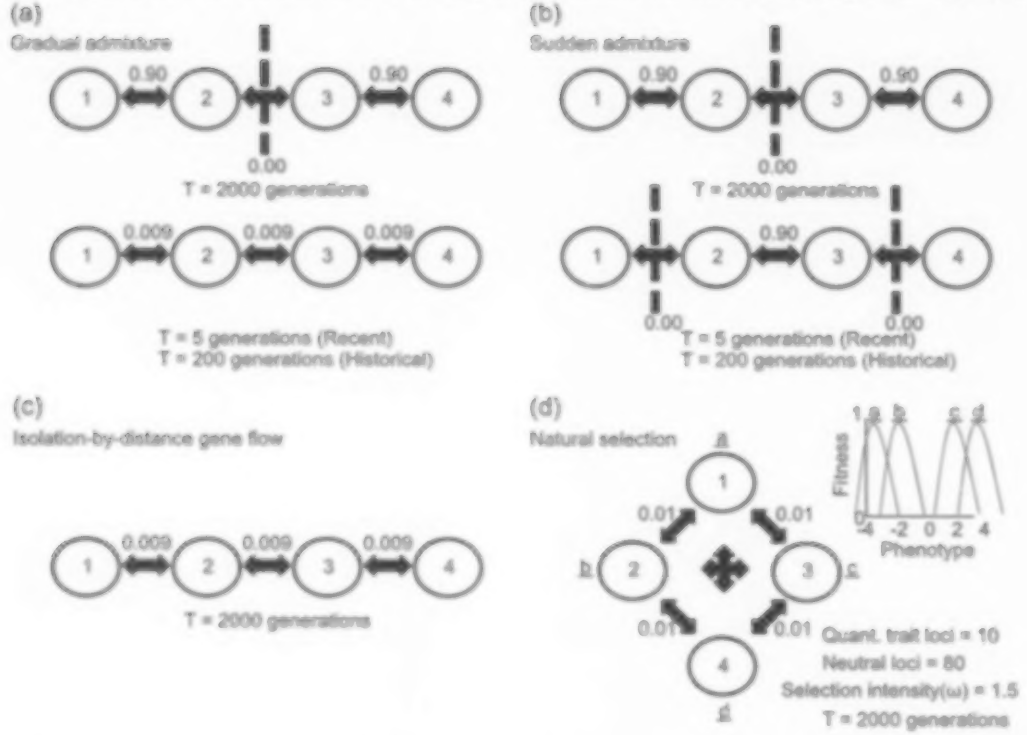
We used the software EASYPOP v. 2.0.1 (Balloux 2001) to generate genotypic data produced by two main models of migration: a contact-zone (admixture) model and a stepping-stone model. Variations of these models were used to simulate a total of five scenarios: (*i*) recent–sudden admixture, (*ii*) recent–gradual admixture, (*iii*) historical–sudden admixture, (*iv*) historical–gradual admixture, and (*v*) isolation-by-distance gene flow. A diagram of the basic characteristics distinguishing each scenario is shown in Fig. 2. For all five scenarios, we used the infinite-allele mutation model at both a fast and slow rate of μ (5×10^{-4} and 2×10^{-5}, respectively) and modeled diploid organisms. Each simulation was initially run for 2000 generations to allow populations to reach the level of drift, migration, and mutation equilibrium given the approximate length of time that may have passed before all Columbia River Basin tributaries would have returned to suitable conditions for salmon habitat following the last glacial maximum. For each scenario, a data set of individual genotypes was generated, each consisting of 90 SNP-like loci that had two possible allelic states.

The main parameter settings were dependent on the scenario being tested and included variable numbers of populations (populations = 4 or 6, total individuals per population = 100 or 50, respectively), rates of migration (sudden and gradual, m = 0.9 and 0.009, respectively), migration schemes, and numbers of generations that passed after migration scheme changed (generations = 200 or 5 for historical or recent admixture, respectively). (A procedural guide describing the specific settings used in EASYPOP simulations for each scenario is provided in online Supplemental Table S2[1].) For example, the "sudden" admixture simulations are characterized by a hierarchical island migration scheme consisting of two groups of three populations each (n = 50 per population), which essentially behaved as two completely isolated populations (migration rate was m = 0.9 and 0.0 for within- and between-groups, respectively). This setting was followed by a second migration scheme in which the two groups (three populations per group) became three groups (two populations per group), effectively allowing the previously isolated populations to interbreed at a high rate (m = 0.9) in one of the groups. This second migration scheme was allowed to proceed for 200 and 5 generations for the historical and recent admixture scenarios, respectively. Alternatively, the "gradual" admixture simulations employed a hierarchical stepping-stone model that isolated two groups of two populations (m = 0.0 between groups) for 2000 generations and changed to a one-dimension stepping-stone migration scheme (m = 0.009 among all populations) to allow a more gradual rate of gene flow to mix the isolates. Finally, the isolation-by-distance gene flow scenario employed a one-dimension stepping-stone migration scheme across four populations (n = 100). Maintaining a migration rate of 0.009 for 2000 generations was found to result in a maximum F_{ST} of 0.4 between two populations at the extremes. The values used for these parameters were chosen to reflect the level of genetic distance observed between interior ocean-type versus interior stream-type Chinook lineages. For all our simulated data sets, the most genetically divergent populations served as the parental categories and the intermediate populations as the test category in NEWHYBRIDS analyses.

To test for effects of natural selection on perceived introgression, we simulated populations of individuals genotyped at 90 loci using the program quantiNEMO v. 1.0.3 (Neuenschwander et al. 2008). In simulation settings, we designated 10 loci as quantitative markers under selection for a single phenotype, and the remaining 80 loci were designated as neutrally evolving. Four populations, "patches", were created with 500 individuals each and allowed to evolve under four different stabilizing selection optima (optimal phenotype values of –4, –2, 2, 4), each with a selection intensity (ω) = 1.5 and a randomly mating island model migration scheme. The value of ω we chose was similar to the value used in

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-107.

Fig. 2. Illustration of the EASYPOP and quantiNEMO simulation settings used for the evolutionary processes examined in this study (i.e., recent and historical admixture, isolation-by-distance gene flow, and natural selection). Panels (*a*) and (*b*) illustrate gradual and sudden rates (T = time) of admixture, respectively, which were examined for both recent and historical admixture scenarios. Bidirectional arrows and corresponding numbers indicate migration rates between populations (circles). Vertical dashed lines represent isolation of populations, which applies only to the admixture scenarios; in each scenario a period of isolation (T = 2000 generations) was followed by gene flow. The natural selection scenario (panel *d*) shows the selection curves (a–d) acting on 10 quantitative trait loci in the four populations. See text for details.



simulations modeling selection of hatchery-reared salmonids ($\omega^2 = 2$; Araki et al. 2008) and also similar to meta-analysis of natural selection studies on wild populations (modal value $\omega^2 = 3.21$; Estes and Arnold 2007). The force of selection in our simulation changed allele frequencies at the ten quantitative trait loci and resulted in two populations that were most genetically divergent from each other ($F_{ST} = 0.53$) and two populations that were intermediate to these extremes (average $F_{ST} = 0.35$ between intermediate and extremes). (Details for the simulation settings can be found in Supplemental Table S3[1].) The two "extreme" populations were used as the pure parental classes and the intermediate-selected populations were classified as test collections for analysis in the program NEWHYBRIDS as described above.

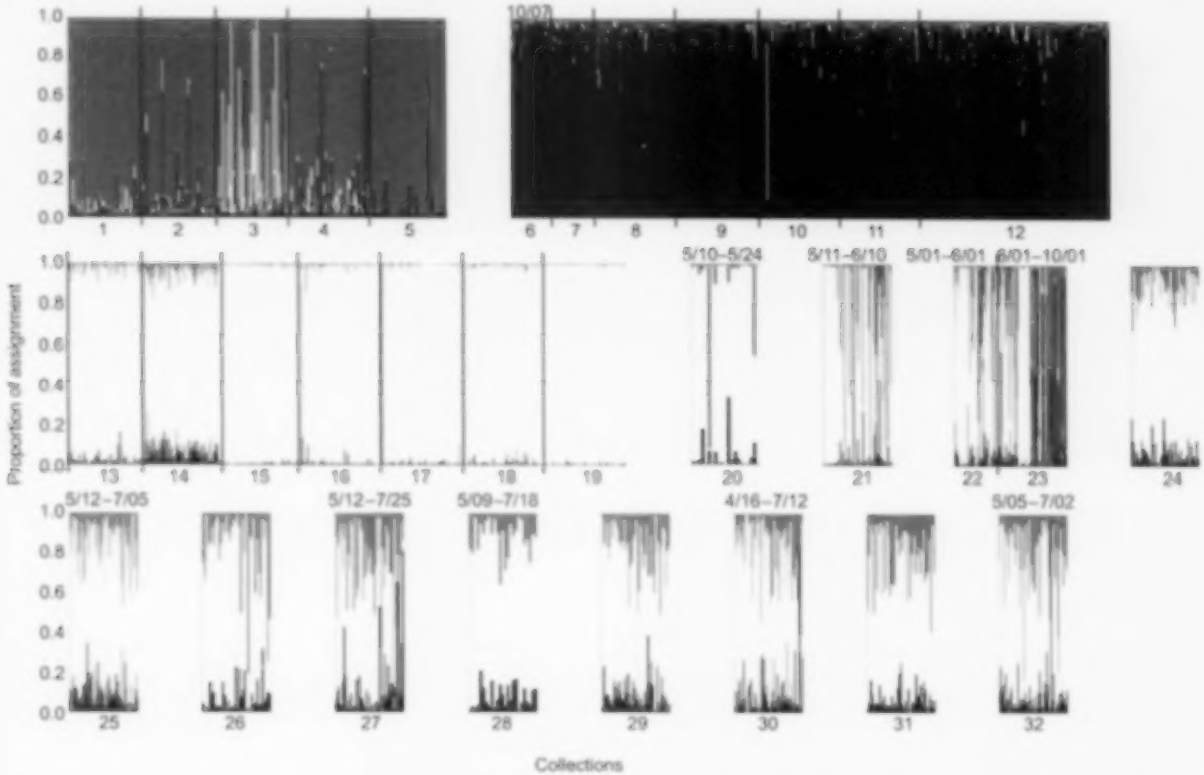
Results

Statistical analyses

Nomenclature for the KRSC collections uses "Klickitat", followed by either "su" or "sp" for summer- or spring-run, respectively. Also, H, NOR, and BRST refer to hatchery, natural origin, and broodstock, respectively, and a two-digit year indicates when the fish were collected. Only the Klickitat_su_85 collection was observed to have more than one locus with significant positive F_{IS} values (Bonferroni-corrected alpha level 0.05 for 94 loci and 32 collections: 1.66×10^{-5}). The only two loci that were found to deviate from HWE in more than one population were *Ots_C3N3* and *Ots_97077-179R*, which deviated in 30 and 7 collections, respectively. *Ots_C3N3* is haploid and was expected to deviate because HWE tests assume diploid markers. *Ots_97077-179R* deviated only within KRSC collections (Klickitat_spBRST_06, Klickitat_spNOR_06, Klickitat_spH_07_08, Klickitat_spNOR_07, Klickitat_spBRST_08, and Klickitat_spNOR_08) and one collection from the Methow River.

The linkage disequilibrium tests revealed 335 pairs of loci with significant linkage within collections (adjusted Bonferroni alpha level 0.05 for 94 loci and 32 collections: 1.66×10^{-5}). The following three pairs of loci were linked in a third or more (>10) of the 32 collections: *Ots_FGF6A* and *Ots_FGF6B_1*, *Ots_hsc71-3'-488* and *Ots_hsc71-5'-453*, and *Ots_PGK-54* and *Ots_RFC2-558*. For each pair of linked loci, we excluded the locus having the lowest minor allele frequency (*Ots_FGF6A*, *Ots_hsc71-5'-453*, and *Ots_RFC2-558*). No other pairs of loci were linked in more than four collections. The numbers of significantly linked locus pairs were not evenly distributed across the 32 collections. In fact, four collections had more than ten significant linked pairs and represented 90% of all significant linkage tests (Table 3). These collections were Kalama (18 pairs), Klickitat_spH_84

Fig. 3. STRUCTURE results for all Chinook salmon collections using $K = 3$ clusters. Corresponding numbered collections are described in Table 3. Date ranges are shown above the collections of Klickitat River Chinook salmon for which run-timing information was known. In these cases, individuals are ordered from earliest to latest date of return. The three major lineages — Lower Columbia, interior ocean-type, and interior stream-type — are represented in grey, black, and white shading, respectively.



(72 pairs), Klickitat_spH_85 (18 pairs), and Klickitat_su_85 (178 pairs); the three Klickitat River collections were also the only collections with significant deviations from HWE based on F_{IS}. Based on these results, we used a reduced panel of 91 independent SNP loci (having omitted three loci in linkage pairs) for all subsequent analyses.

Results from STRUCTURE that quantify lineage membership demonstrated that some of the collections with the most extreme departures from HWE were also found to have the most stray individuals from other lineages. The ten STRUCTURE trials ($K = 3$) produced a final optimized pairwise similarity (H') score of 0.997. In general, collections were found to be predominantly composed of individuals that strongly assigned ($Q > 80\%$) to one of the three STRUCTURE clusters (Table 3; Fig. 3), and results were concordant with expected membership of these baseline collections to the three major genetic lineages (Lower Columbia, interior ocean-type, and interior stream-type) based on previous studies (Narum et al. 2010). Moreover, most baseline collections showed less than 10% average assignment to a cluster different from their predominant lineage, except the Kalama River collection, which had 17% assignment to the interior stream-type lineage (Table 3). The KRSC test collections were also exceptional, because although the interior stream-type lineage was predominant, all collections had greater than 10% average assignment to a non-interior-stream-type lineage. In some of these collections, both interior ocean-type and Lower Columbia lineages had greater than 10% average assignment. Evaluation of collections at the individual level showed 11 collections contained at least one "stray" individual, which we defined as having greater than 80% (Q) to a cluster different from the putative lineage-of-origin for the collection (as indicated by the majority of individuals; Table 4). We excluded a total of 55 strays (2% of the data set), because they were unlikely to have contributed to the current genetic composition of the breeding populations for which we wanted to quantify introgression and would have biased our subsequent analyses. After excluding strays from the data set, the collections were tested again for HWE deviations based on linkage disequilibrium among locus pairs and on F_{IS} values and calculated for average STRUCTURE assignment (Table 4). The numbers of loci with significant HWE deviations within collections were fewer compared with previous estimates; however, there were two collections that continued to have large numbers of locus pairs in linkage disequilibrium and significantly positive F_{IS} values. Both were KRSC collections (Klickitat_spH_84 and Klickitat_su_85). All 19 baseline collections were found to have greater than 80% average assignment to their putative lineage-of-origin, as well as three KRSC collections (predominantly interior stream-type lineage). We used the reduced data set of 2358 individuals (excluding 55

Table 4. Chinook salmon collection descriptions after removal of assigned stray fish from collections.

	Strays				New assignment			Descriptive statistic		
Collection ID	LC	OT	ST	N^a	LC	OT	ST	P	New F_{ST}	LD
1. McKenzie	0	0	0	84	**0.93**	0.04	0.02	0.7328	–0.009	0
2. Cowlitz	0	0	0	86	**0.92**	0.06	0.02	0.5731	–0.003	0
3. Kalama	0	0	5	76	**0.81**	0.07	0.13	0.2939	0.008	4
4. Lewis	0	0	0	93	**0.87**	0.09	0.04	0.0283	0.024	0
5. Spring Creek	0	0	0	88	**0.96**	0.04	0.00	0.7381	–0.009	0
6. Klickitat_fa_99	0	0	0	45	0.05	**0.94**	0.01	0.0992	0.025	0
7. Klickitat_fa_04	1	0	0	46	0.04	**0.94**	0.01	0.1937	0.017	0
8. Lower Deschutes	0	0	0	90	0.10	**0.89**	0.01	0.0065	0.034	0
9. Hanford	0	0	0	93	0.04	**0.95**	0.01	0.2490	0.009	1
10. Wells Dam	0	0	0	89	0.04	**0.94**	0.03	0.0075	0.034	0
11. Lyons Ferry	0	0	0	90	0.07	**0.92**	0.01	0.0278	0.027	1
12. Clearwater	0	0	0	213	0.07	**0.92**	0.01	0.9995	–0.029	1
13. John Day	0	0	0	84	0.02	0.02	**0.95**	0.1174	0.017	0
14. Cle Elum	0	0	0	88	0.05	0.07	**0.88**	0.0310	0.025	0
15. Wenatchee	0	0	0	85	0.01	0.01	**0.99**	0.1748	0.014	0
16. Methow	0	1	0	92	0.01	0.01	**0.98**	0.0106	0.032	0
17. Imnaha	0	0	0	92	0.01	0.01	**0.98**	0.6021	–0.004	0
18. Lolo	0	0	0	89	0.01	0.01	**0.98**	0.3456	0.006	1
19. Big Creek	0	0	0	92	0.01	0.01	**0.99**	0.4217	0.003	5
Klickitat (KRSC)										
20. spH_80	1	0	0	20	0.04	0.05	**0.91**	0.0461	0.049	0
21. spH_84	7	0	0	53	0.14	0.04	**0.81**	**<0.0001**	0.068*	19
22. spH_85	1	1	0	52	0.17	0.07	0.76	0.0050	0.044	6
23. su_85	27	5	0	51	0.27	0.12	0.60	**<0.0001**	0.137*	25
24. spH_02	0	0	0	47	0.11	0.07	**0.82**	0.4605	0.002	0
25. spH_06	0	0	0	85	0.14	0.10	0.76	0.1807	0.012	0
26. spBRST_06	1	0	0	37	0.16	0.08	0.76	0.3124	0.009	0
27. spNOR_06	1	1	0	48	0.23	0.13	0.64	0.0052	0.045	1
28. spH_07_08	0	0	0	25	0.12	0.08	0.80	0.0297	0.047	0
29. spBRST_07	0	0	0	46	0.18	0.10	0.72	0.0152	0.038	0
30. spNOR_07	0	1	0	76	0.19	0.09	0.72	0.0006	0.045	1
31. spBRST_08	0	0	0	45	0.22	0.07	0.70	0.0028	0.051	0
32. spNOR_08	1	1	0	58	0.22	0.08	0.70	0.4758	0.001	1

Notes: Stray fish were defined as those having greater than 80% STRUCTURE assignment (Q) to a cluster different from the putative lineage-of-origin (see Table 3). Adjusted sample sizes (N^a) reflect exclusion of strays from collections; total sample size (N^a) is 2358. New assignments based on N^a and adjusted descriptive statistics are defined as described in Table 3. Bolding under new assignments indicates >80% membership to a particular STRUCTURE cluster; under descriptive statistics, significant results are indicated with bold font and also with an asterisk (*).

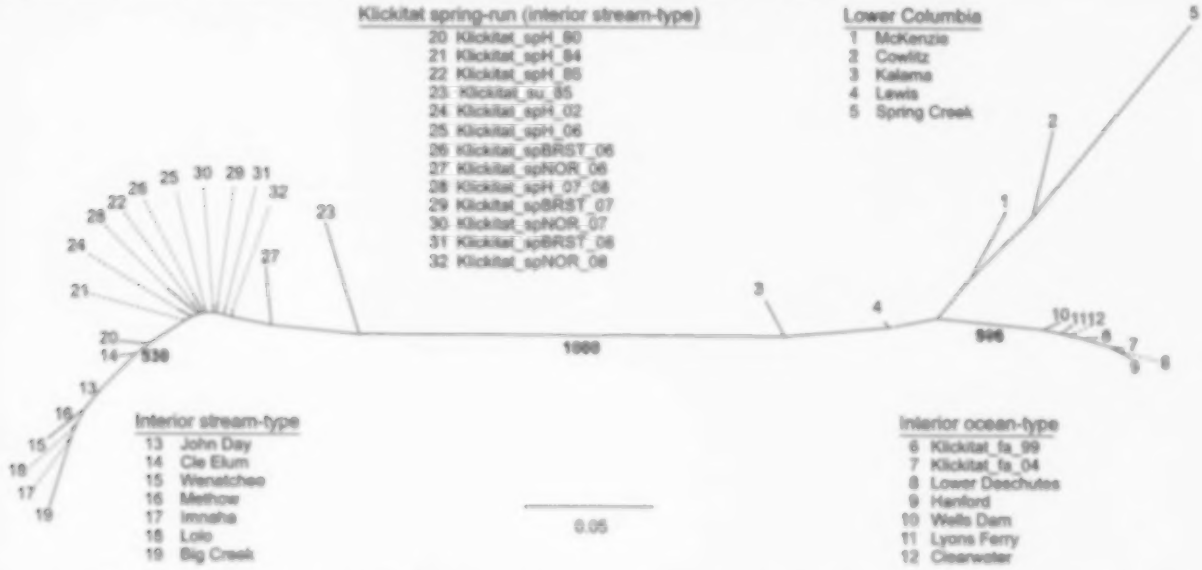
strays) to examine phylogenetic relationships among collections and quantify introgression within collections.

A NJ dendrogram based on pairwise Cavalli-Sforza and Edwards (1967) chord distance among collections shows a relatively large genetic distance between the interior stream-type lineage and the collections representing the Lower Columbia – interior ocean-type Chinook salmon lineages of Columbia Basin (Fig. 4). There is high bootstrap support (90.6% based on the consensus tree) for the branch node separating the Lower Columbia and interior ocean-type collections. Further, there is 100% bootstrap support for the node separating all interior stream-type collections, including the KRSC, from the other two lineages. Although KRSC shows greatest genetic similarity to the interior stream-type lineage, it is important to note that the KRSC collections remain outside the branch occupied by all the reference interior stream-type collections, which had 53.0% bootstrap support.

Pairwise F_{ST} values among interior stream-type and KRSC collections were used to examine evidence for isolation-by-distance gene flow (matrix available in online Supplemental Table S1[1]). Regression of genetic distance (F_{ST}) with stream distance (miles) indicated no significant correlation for this IBD analysis.

The following nine loci were determined to be diagnostic for distinguishing interior stream-type versus interior ocean-type – Lower Columbia lineage groups: *Ots_nramp-321*, *Ots_cox1-241*, *Ots_FARSLA-220*, *Ots_HSP90B-100*, *Ots_Raros-250*, *Ots_P450*, *Ots_SL*, *Ots_u07-57.120*, and *Ots_ZR-575*. We considered loci diagnostic when these two lineage groups were nearly fixed for alternate alleles. Specifically, "near fixation" was defined by having 95% of the posterior probability densities contained within an allele frequency range of 0.0–0.2 and 0.8–1.0,between the two different lineage groups. If these allele frequency bounds are relaxed to the ranges of 0.0–0.3 and 0.7–1.0, the following 14 loci qualify as diagnostic: *Ots_nramp-321*, *Ots_ARNT*, *Ots_*

Fig. 4. Phylogenetic relationships of Columbia River Chinook salmon collections. A neighbor-joining tree was constructed using Cavalli-Sforza and Edwards (1967) chord distance. Bolded values indicate results of 1000 bootstraps. Collections are listed in four categories, which include the three major lineages in the Columbia River Basin and the test collections composed of Klickitat River spring-run Chinook salmon. Dashed lines connect collection labels to their position on the tree for clarity around dense nodes.



cox1-241, *Ots_ETIF1A*, *Ots_FARSLA-220*, *Ots_hsc71-3-488*, *Ots_HSP90B-100*, *Ots_Ikaros-250*, *Ots_MHC1*, *Ots_P450*, *Ots_RAG3*, *Ots_SL*, *Ots_u07-57.120*, and *Ots_ZR-575*.

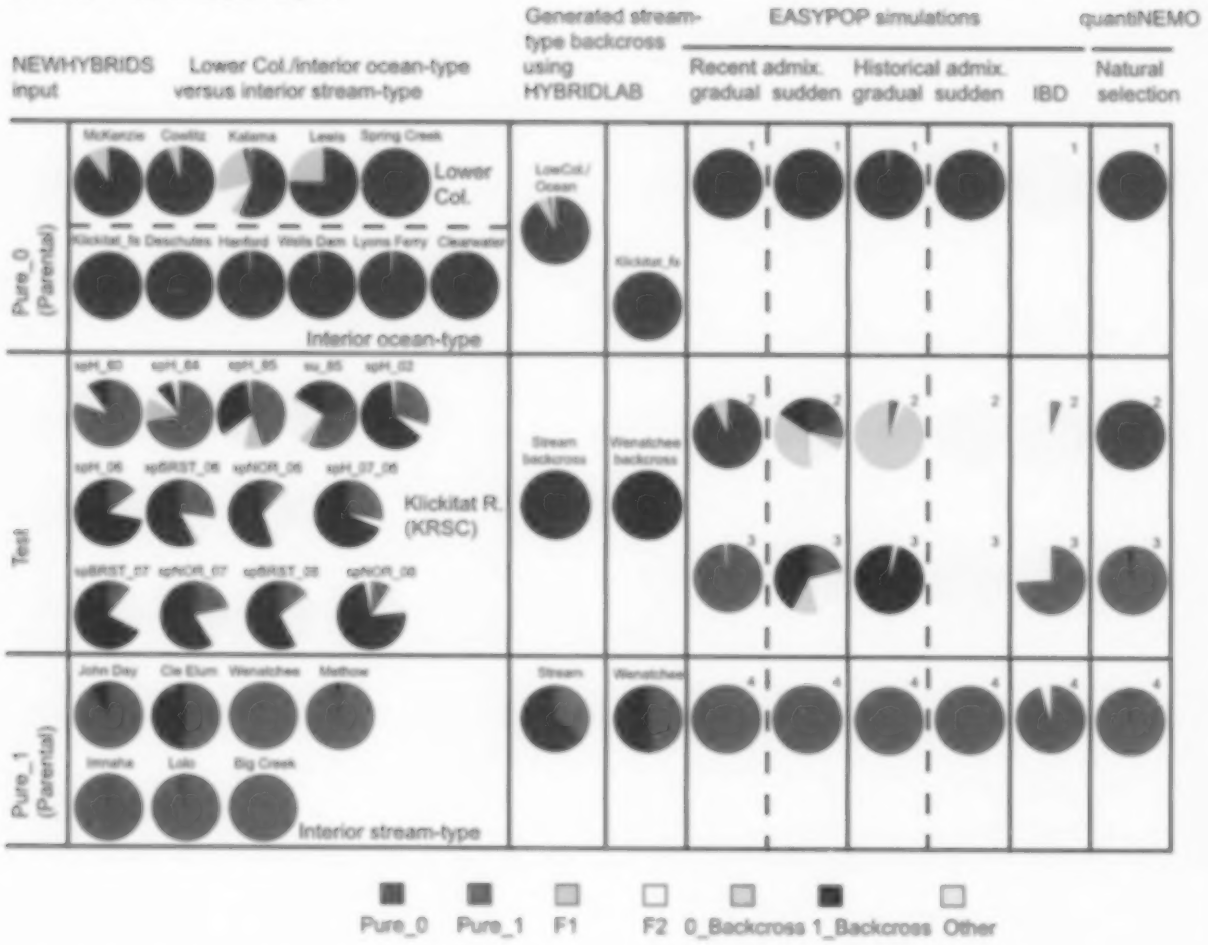
Results from NEWHYBRIDS indicated a majority of individuals from most of the KRSC collections assigned to the genotype class described as a backcross to the pure_1 parental population (interior stream-type lineage). KRSC collections from 1980 to 1985 were the exceptions to this general result; these collections had large proportions of individuals assigning to the pure_1 parental genotype class. These patterns changed little regardless of which collections were used to represent the pure_0 parental population (only interior ocean-type, only Lower Columbia, or interior ocean-type and Lower Columbia collections combined). Therefore, given the program constraint of two parental types, we have only reported results from simulations using pure_0 parental (from combining interior ocean-type and Lower Columbia lineages) and pure_1 parental populations (Fig. 5). The temporal differences in genetic composition among the KRSC collections were quantified by plotting the average individual assignment to the pure_1 parental genotype class across years (Fig. 6). This analysis shows a 20% decline in average individual assignment between estimates of collections from 1984 and 1985, followed by another 30% decline represented by estimates of collections from 2002 to 2008. This pattern of decline was almost evenly matched by a pattern of increase of the 1_backcross genotype class. We also compared genetic composition of fish returning only in the month of May across different years in the Klickitat River, which was intended to remove any bias that could be introduced from comparing fish from different years that were collected over slightly different times in the season (i.e. containing fewer or greater numbers of summer- and fall-run fish). A shift in genetic composition of May-returning fish over time in years was similar to the shift observed in comparisons of total collections (Fig. 6). Potential effects of full-sibship were examined by including only one individual from each putative full-sibling family and re-analyzing estimates of introgression in KRSC. There was no strong correlation of previous tests of linkage and numbers of full-sibling pairs among these collections (range of 2–17 full-sibships). Even when accounting for full-sibling families by excluding all members except one representative from each full-sibling family (n = 105 excluded), the pattern of a dramatic shift of KRSC introgression through time (Fig. 6) remained unchanged.

Aside from results for the KRSC "test" collections, we observed a few noteworthy patterns among the baseline "parental" populations. Most collections were composed of individuals from a pure parental genotype class, but the Kalama, Lewis, and Cle Elum collections had large percentages (>25%) of backcross individuals. This result is concordant with the STRUCTURE analysis, which found relatively high average individual assignments of 13% interior stream-type, 9% interior ocean-type, and 12% combined interior ocean-type – Lower Columbia for the Kalama River, Lewis River, and Cle Elum River collections, respectively (Table 4).

Simulations

The validity of the observed pattern of interior stream-type (pure_1 parental) backcrosses in our KRSC collections was examined using HYBRIDLAB. This program was in fact able to reproduce a set of simulated individual genotypes that were indicated as interior stream-type backcrosses using the program NEWHYBRIDS (Fig. 5). However, the randomly drawn individuals used to compose parental populations for use in HYBRIDLAB did not produce a reasonably pure interior stream-type parental genotype class. When we

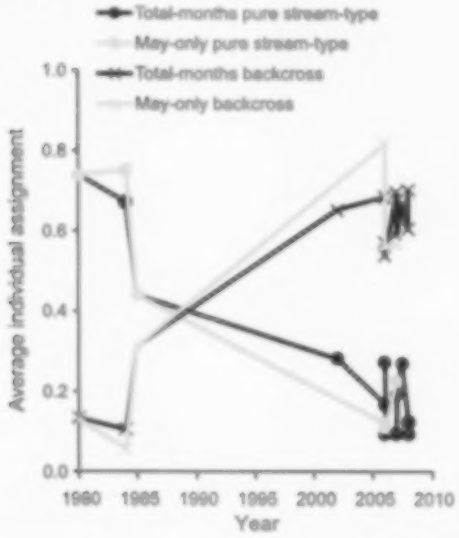
Fig. 5. NEWHYBRIDS individual assignment to six genotype classes. Each pie chart shows the proportion of individuals from a collection assigning with greater than 50% probability to one of six genotype classes (corresponding colors are labeled at bottom). The "other" category captures any individuals failing to assign to any of the genotype classes. The results of the empirical data set are shown on the left and all results from the simulations discussed in the Materials and methods are shown on the right. Under HYBRIDLAB, pure_0 and pure_1 were chosen from the baseline reference collections, and the middle row chart is a product of the backcross simulation. The EASYPOP and quantiNEMO simulations were designed to yield two divergent parental populations (labeled 1 and 4) and two populations that were genetically intermediate between the parental populations. In each case, the simulated population 3, which was more genetically similar to pure_1 parental, was compared with the KRSC collections in the empirical data set. Population numbers for these simulations correspond with those used in the simulation diagram (Fig. 2).



used a single collection each from the Klickitat River fall-run and Wenatchee River spring-run to represent the pure parental populations, this problem was less pronounced; however, the Wenatchee pure_1 parental population still appeared to have a greater proportion of interior stream-type backcross individuals than was observed for that collection in the analysis of the total empirical data set.

The simulations that were used to model evolutionary processes of recent and historical admixture, isolation-by-distance gene flow, and natural selection were evaluated for their ability to reproduce the patterns we observed in the empirical data set. Specifically, which of the simulation scenarios could produce collections that were predominantly composed of individuals assigning to the pure_1 parental backcross genotype class prevalent in the KRSC collections? The EASYPOP and quantiNEMO simulations produced two collections (labelled 2 and 3: Fig. 5) that were intermediate in genetic distance between the pure parental populations. For comparisons with KRSC, we focused on the collections labelled as 3 from these simulations, because they are more genetically similar to pure_1 parental population. Using the fast mutation rate, the following three simulated scenarios produced collections with more than half of individuals assigning to the pure_1 parental (i.e. interior stream-type) backcross: sudden–recent admixture, gradual–historical admixture, and isolation-by-distance gene flow (not shown). The remaining three simulated scenarios (gradual–recent admixture, sudden–historical admixture, and natural selection) resulted

Fig. 6. Change in genetic composition of Klickitat River spring-run Chinook salmon through time. Average assignment to two genotype classes (circles = pure_1 interior stream-type, Xs = interior stream-type backcross) of individuals from Klickitat River spring-run Chinook collections are plotted by year. Subsets of fish from each collection that were collected in the month of May are shown in grey. Black lines indicate total collections regardless of which month fish were collected.



in collections with fewer than 10% pure_1 interior stream backcrosses. Of these latter three scenarios, the sudden–historical admixture scenario was unique in that it resulted in collections that were 100% composed of individuals assigning to the F_2 genotype class. Use of the slow mutation rate significantly changed the results of only one of the simulations, the isolation-by-distance scenario, which no longer contained interior stream-type backcrosses. Instead, this scenario resulted in a collection predominated by pure_1 parental individuals and a portion of F_2 individuals. The results from the slower mutation rate are likely more realistic because that rate is more characteristic of SNPs (e.g., Kruglyak and Nickerson 2001). All the results using this slow rate are displayed (Fig. 5).

Discussion

Processes that led to introgression of Klickitat River spring-run Chinook salmon

Our analyses indicate that the Klickitat River spring-run Chinook salmon (KRSC) may have historically been more closely related to other interior stream-type representatives of the Columbia River, but within the past 30 years has experienced introgression from a recent admixture event related to hatchery practices. This conclusion was based on both our analysis of computer simulations of four different processes (recent and historical admixture, isolation-by-distance gene flow, and natural selection) and empirical analysis of the genetic composition of the KRSC collections in relation to our baseline samples from the Columbia River Basin. Using simulations of four processes, we could exclude isolation-by-distance gene flow and natural selection because these processes had the least potential to result in the patterns of introgression that were observed in the KRSC collections. Isolation-by-distance gene flow also did not appear to fit well with our empirical data set based on regressions of geographic distance (stream distance) with genetic distance (pairwise F_{ST}) using all interior stream-type and KRSC collections. Therefore, recent and historical admixture events were the most likely remaining alternative hypotheses that required further evaluation. We concluded that recent admixture had the most support based on a temporal series of KRSC collections that spanned 30 years from the early 1980s to the 2000s, because the composition of the KRSC was found to shift within this time frame from predominantly interior stream-type pure parental genotypes to predominantly interior stream-type backcrossed genotypes. If the genetic composition of these collections had not been found to change over this 30-year span we examined, we would have been unable to reject either recent or historical admixture as alternative hypotheses.

The shift in genetic composition of KRSC appears to have begun after 1984 and continued to shift between 1985 and 2002. Chinook salmon are generally 4 years old by the time they return as adults to spawn in their natal streams, but can also mature earlier as 3-year-olds (Quinn 2005). Therefore, the first group of introgressed mature Chinook salmon would have resulted from spawning that occurred around 1980. Further, changes in hatchery practices that could have led to non-interior-stream-type Chinook salmon being incorporated into broodstock used for the KRSC must have occurred 2–4 years before 1980. This timing may implicate one particular change in hatchery practices in which a stock of interior ocean-type summer–fall-run Chinook salmon from the Upper Columbia River was released as yearlings in 1977. When some of these yearlings returned to spawn in 1979, they could have been collected as broodstock and crossed with the indigenous interior stream-type fish that comprised the spring run, and their F_1 offspring would have begun returning in 1984. The F_1 offspring could then continue to be introgressed with this breeding aggregate through natural or human-induced backcrossing and continued plantings of interior ocean-type stocks. The timing of the shift of genetic composition observed in the KRSC may preclude the role of all other earlier hatchery events (e.g., release of Lower Columbia spring-run fish), because these events would have substantially altered genetic composition prior to the earliest collections that were examined in this study.

If hatchery practices were responsible, the question remains as to why we observe a shift in genetic composition after 1985, despite the fact that fall- and spring-run salmon from the Lower Columbia lineage had been planted in the Klickitat River from 1946 to 1986 with seemingly minimal effect on the genetic composition of the spring run. One reason may be due to two major differences between the Lower Columbia fall-run and Upper Columbia River interior ocean-type stocks that were introduced to the Klickitat River at separate points in time. The stocks differ both morphologically and in run timing. These differences make it more challenging for human observers to distinguish the Upper Columbia River interior ocean-type stock from the KRSC stock, which appear more similar morphologically and have greater tempo-

ral overlap in run timing (i.e. bright coloration, summer migration) compared with the Lower Columbia stock (i.e. dark coloration, fall migration). Therefore, when choosing hatchery broodstock, hatchery operators rely primarily on run timing and use morphological cues to avoid interbreeding nonindigenous stocks. This strategy appears to have maintained a pure interior stream-type broodstock in the spring-run hatchery program during the years when a morphologically distinguishable Lower Columbia fall run was present; however, these phenotypic traits appear to have been insufficient for maintaining separation from the interior ocean-type summer–fall-run fish from the Upper Columbia River. There is also evidence from the Klickitat Hatchery records that on at least a few occasions, fish that may have been summer- or early fall-run Chinook salmon were incorporated into KRSC broodstock collections. These fish returned (volunteering into hatchery holding ponds via an adult fish ladder) and sexually matured at a later date than most of the KRSC, but enough overlap in this timing was present to provide for potential interbreeding.

Consequences of genetic exchange between hatchery and wild populations of salmonids

The potential for negative genetic effects of hatcheries on wild populations of salmonids has been discussed for a variety of salmonid species (e.g., Waples 1991; Jonsson and Jonsson 2006; McClure et al. 2008). The outbreeding depression that can result from mixing of highly genetically divergent lineages is perhaps one of the more extreme examples of these genetic interactions. However, evidence for negative fitness effects of introgression of hatchery strains and wild populations has been demonstrated in cases in which the genetic distance between the hatchery strain and natural population was much smaller than the distance among lineages of Chinook salmon examined in this study (e.g., in steelhead (*Oncorhynchus mykiss*) McLean et al. 2004; and brown trout (*Salmo trutta*) Hansen et al. 2009). In such cases, domestication selection of the hatchery strain may be the reason for its lower fitness relative to the natural population, and the sheer volume of hatchery releases can drive introgression of hatchery and wild fish in nature. In contrast, our study may illustrate an example of introgression perpetuated mainly through direct anthropogenic influence. Evidence from the Snake River Basin suggests that there are strong reproductive isolating mechanisms that maintain the interior ocean-type and the interior stream-type Chinook salmon as distinct genetic lineages in naturally spawning populations (Narum et al. 2007). There is also support that these reproductive isolating mechanisms are prezygotic and largely driven by the selective advantage of these lineages in the different stream environments in which they spawn and migrate (Brannon et al. 2004), as well as the differing ocean environments preferred for feeding grounds (Trudel et al. 2009; Weitkamp 2010). In fact, Waples et al. (2004) suggest that the interior ocean- and stream-type lineages in the Columbia River are effectively behaving as separate biological species in sympatry. Matala et al. (2011) used the same SNP loci from this study and showed evidence of local adaptations within populations distributed throughout the Columbia River Basin, which was based on significant correlations among subsets of SNP loci with environmental variables (e.g., elevation). Therefore, it is likely that under natural conditions, even sympatric populations of interior stream- and ocean-type fish (e.g., within the Klickitat River subbasin) would remain genetically distinct through time. However, through human intervention and manipulation these lineages are capable of successful reproduction and persisting as an introgressed population at least with continued supplementation from a hatchery program.

It is not clear whether a shift in genetic composition has led to any life history or phenotypic changes, but there is some evidence that the KRSC retain many of the characteristics of other interior stream-type populations. For example, KRSC characteristics include yearling smolt migration, limited recoveries of coded wire tags from coastal fisheries, and comparable vertebral counts with interior stream-type Chinook salmon (reviewed in Myers et al. 1998). However, even if there is a negative fitness cost associated with KRSC introgression, perhaps simply minimizing human influences and allowing populations to re-establish natural reproductive isolation may be a viable solution, which has been pursued in other species (e.g., McLean et al. 2004).

Technical challenges involved with intraspecific hybrid detection

Our data set demonstrated high power for use in hybrid detection analyses. As few as 12 microsatellite loci with pairwise F_{ST} between hybridizing parental populations of 0.21 have been shown to be required for efficient detection of F_1 hybrid individuals using STRUCTURE and NEWHYBRIDS (Vähä and Primmer 2006). In our case, we had many loci (91 and 90 loci for STRUCTURE and NEWHYBRIDS, respectively) with high F_{ST} between hybridizing parental populations (approximately 0.4). Since Vähä and Primmer (2006) employed microsatellites, it may be more appropriate to compare these genetic markers in terms of independent alleles (i.e. 72 independent microsatellite alleles; 12 loci and 6 independent alleles per locus) versus 90 independent SNP alleles. This comparison of number of independent alleles demonstrates the high efficiency of our markers, and this power was likely further enhanced by the fact that 9–14 of these loci were diagnostic for the parental populations. Intraspecific hybridization can impose a substantial technical challenge for hybrid detection, but with the increasing availability of SNP markers, sufficient numbers of diagnostic loci will likely be achievable for a range of questions related to introgression of hatchery and wild fish populations.

One of the parental populations in this study, "Lower Columbia – interior ocean-type", contained substructure because we combined two lineages together. This combination has the potential to create problems when using the program NEWHYBRIDS because it was designed to accept only two diverged parental groups. This combination did not appear to lead to spurious results because analyses using either the Lower Columbia or interior ocean-type collections separately as the pure_0 parental population produced similar proportions of genotype classes in our test KRSC collections (not shown). Results are shown with HYBRIDLAB, in which we generated a simulated interior stream-type backcross population with the following two choices for a pure_0 parental population to cross against a representative interior stream-type collection: (*i*) randomly sampled individuals from both

Lower Columbia and interior ocean-type collections or (*ii*) a single interior ocean-type representative (Klickitat River fall-run). Both simulations produced individuals identified as interior stream-type backcrosses. Further, although introgression in the KRSC collections could have potentially involved all three major lineages, the timing of this shift in genetic composition occurred during a major change in hatchery practices involving only the interior ocean-type lineage. Therefore, we feel justified in using NEWHYBRIDS analyses despite having three lineages present in this study; two of these lineages are distinct but relatively closely related (Lower Columbia and interior ocean-type), and the third lineage is much more genetically distant (interior stream-type).

Management implications for the Klickitat River subbasin and beyond

A series of reforms to Klickitat Hatchery practices are planned, including changing the location and method of broodstock collection, tracking individual fish collected via tagging technologies, and possible genetic assessment of fish prior to actual broodstock selection. These actions may inhibit further introgression and help alleviate any potential population-level effects; however, it is possible that introgression and any associated effects are essentially irreversible. Chinook salmon constitute a metapopulation and may require stock transfers from nearby mid-Columbia River populations of more pure interior stream-type Chinook salmon to be incorporated in the broodstock to move the KRSC back towards a pure stock.

Introgression detected in other Chinook salmon populations will also likely need to be addressed. A study by Narum et al. (2010) has revealed other examples of introgression in collections within each of the Chinook salmon lineages in the Columbia River Basin. Our study also showed substantial hybridization occurred in the Lower Columbia River and a subbasin in the Middle Columbia River. In the Lower Columbia River, both the Lewis River fall-run and the Kalama River spring-run collections appear to have been introgressed with Lower Columbia and interior ocean-type and Lower Columbia and interior stream-type, respectively. In both cases, hatchery planting of out-of-basin stocks may explain these patterns (Myers et al. 2006). Perhaps more surprising was detection of introgression in the Cle Elum spring-run population from the Yakima River subbasin in the Middle Columbia River. This collection represents one of the rare populations that maintain substantial Chinook salmon run sizes in the Middle Columbia River spring-run evolutionarily significant unit (Myers et al. 1998). Hatchery supplementation was previously thought to have had negligible influence on the native runs of spring Chinook in the Yakima (Marshall et al. 1995). Our results suggest genetic monitoring using highly informative markers for hybrid detection of all populations, even those viewed as fairly pristine, may be useful to identify problems early and avoid extensive admixture observed in the KRSC.

Despite the challenges involved in studying intraspecific hybridization, we have demonstrated an ability to detect conspecific hybrids and discriminate among natural and anthropogenic processes that may have caused the mixed ancestry of a population. Our use of archived scales from the 1980s made it possible to conclude a recent anthropogenic admixture event was the most likely explanation and thus underscores the importance of archived tissues to gain insight into contemporary observations. Further investigation is needed to determine how introgression affects the fitness of this population and to choose the most appropriate management tactic for mitigation according to the severity of any negative impacts.

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Conflicting objectives in trophy trout recreational fisheries: evaluating trade-offs using an individual-based model

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Abstract: Standard fisheries models, based on average population metrics, are inadequate for analyzing recreational fisheries where fishing is size-selective and management objectives are related to preserving population size structure. We developed a framework for policy analysis of size-based harvest strategies in recreational fisheries. The framework combines a mixed-effects body growth model and an individual-based harvest model to describe the relationship of growth, mortality, and size structure. Fishery performance is quantified with indicators directly associated to catch-related components of anglers' satisfaction: yield (kg), population size, and availability of trophy-size fish. We applied our analyses to the steelhead (*Oncorhynchus mykiss*) fishery in the Santa Cruz River (Patagonia, Argentina). Large declines in trophy-size fish are to be expected at fishing mortalities much too low to cause a sizeable decline in recruitment from virgin values. When somatic growth is density-independent, harvest occurs at the expense of other indicators associated with the quality of fishing experienced by individual anglers. Size limits provide a tool to better accommodate harvest without compromising fishing quality. When preserving population size is favored over preserving trophy-size fish, minimum size limits constitute the best policy overall, whereas maximum size limits are best when the emphasis is on preserving trophy-size fish.

Résumé : Les modèles standards de pêche, basés sur les métriques moyennes de la population, sont inadéquats pour analyser les pêches sportives dans lesquelles la pêche est sélective en fonction de la taille et les objectifs de gestion cherchent à conserver la structure en taille de la population. Nous avons mis au point un cadre pour l'analyse des politiques des stratégies de capture en fonction de la taille dans les pêches sportives. Le cadre combine un modèle à effets mixtes de croissance corporelle et un modèle de récolte basé sur l'individu afin de décrire la relation entre la croissance, la mortalité et la structure en taille. La performance de la pêche est déterminée à l'aide d'indicateurs directement associés aux composantes reliées à la capture dans la satisfaction des pêcheurs sportifs, soit le rendement (kg), la taille de la population et la disponibilité de poissons de taille trophée. Nous avons appliqué nos analyses à la pêche de truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) de la rivière Santa Cruz (Patagonie, Argentine). On peut s'attendre à de forts déclins des poissons de taille trophée à des mortalités dues à la pêche beaucoup trop faibles pour causer une diminution substantielle dans le recrutement par rapport aux valeurs originales. Lorsque la croissance somatique est indépendante de la densité, la récolte se fait aux dépens des autres indicateurs associés à la qualité de la pêche perçue par les pêcheurs sportifs individuels. Les limites de taille représentent un outil pour mieux accommoder la récolte sans compromettre la qualité de la pêche. Lorsqu'on favorise le maintien de la taille de la population plutôt que la préservation des poissons de taille trophée, les limites de taille minimale constituent la meilleure politique globale, alors que les limites de taille maximale sont plus appropriées lorsque l'emphase est sur la préservation des poissons de taille trophée.

[Traduit par la Rédaction]

Introduction

The potential of recreational fishing to adversely impact fish populations has gained recognition in recent years, pointing to the need for more effective management strategies for these fisheries (Post et al. 2002; Cooke and Cowx 2004, 2006). Unlike commercial fisheries, which are commonly assessed and managed by sophisticated methods, specific approaches for recreational fisheries are much less developed. It is not uncommon to import regulations from other recreational fisheries of the same species, with little specific support for them (Redmond 1986; Brousseau and Armstrong 1987; Radomski et al. 2001). There is a clear need for analytic frameworks that recognize the realities and specific management objectives of recreational fisheries.

In developing such frameworks, three specific characteristics of recreational fisheries need to be considered. First, most recreational fisheries are fully open access, with universal rights to buy a license and fish (Cox and Walters 2002). Unlike many commercial fisheries, regulating fishing effort through the number of licenses sold is not an option; realized effort is highly variable and dependent on a combination of factors, such as the interest drawn by the fishery and its ac-

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cessibility as determined by costs, facilities, and geographical location (Cox and Walters 2002). In such fisheries, catches can only be indirectly regulated by setting limits to the operation of individual anglers: creel limits, closed seasons, gear restrictions, area closures, catch-and-release regulations, and size limits (Noble and Jones 1999).

Second, the objectives of recreational fishing are complex and cannot be expressed in terms of maximizing the harvest in kilograms or dollars, but are more closely associated to the overall quality of the fishing experience. We focused our analyses on fisheries such as trout fisheries, where the interest of users is largely directed at entertainment, and quality fishing involves satisfying a diverse array of anglers' desires and expectations (Radomski et al. 2001; Johnston et al. 2010). For instance, in many trout fisheries worldwide, anglers' motivations are often related not only to the quality of the fishing itself (e.g., catch rate or the size of the fish caught), but also to variables associated with recreational value beyond the fish populations, such as scenic value, the quality of lodging, etc. (Vigliano et al. 2000; Hutt and Bettoli 2007). Mapping the perceptions of recreational anglers into quantitative indicators of quality and defining clear management objectives are not easy tasks because solutions depend on the type of anglers involved and their values and motivations (Fedler and Ditton 1994; Peterson and Evans 2003; Johnston et al. 2010).

Third, the expectations of multiple users may be conflicting in nature. Clearly, allowing harvest for those anglers who take their catch home and preserving high catch rates of large fish for those practicing strict catch-and-release fishing are conflicting management goals. A central objective of modern fisheries science is to clearly expose such trade-offs between alternative management objectives, providing managers with tools to decide where to operate along the trade-off (Walters and Martell 2004). The recognition of trade-offs among management objectives is emerging as a major issue in the study of recreational fisheries. As Pitcher and Hollingworth (2002, p. 13) state, "the generally unrecognized reason for failures in sport fishery management….is that there is no Holy Grail of an optimal solution".

The three specific characteristics of recreational fisheries described above have been widely recognized in the specific literature, but formal analyses of trade-offs or general principles for managers to operate within these trade-offs given the regulatory tools at hand do not exist. To provide such an analysis, our first specific objective is to develop different indicators of performance that reflect the diversity of management objectives in recreational fisheries and use a simulation model to explore their response to different management strategies. We combine typical fishery indicators (yield in numbers and in biomass, spawning biomass, recruitment rate) with others more specifically related to the quality of recreational fishing (average fish size, probability of catching a trophy-size fish). Models traditionally used to analyze recreational fisheries regulations consider the average growth of fish (e.g., Clark et al. 1980; Taylor 1981; Jensen 1981). Such models cannot accurately assess the effects of size-selective harvesting on population size structure (Sainsbury 1980; Parma and Deriso 1990). We develop an individual-based model (IBM) of recreational fishing that explicitly incorporates individual variation in growth, therefore allowing a more realistic representation of size-dependent processes in the life history and in the fishery.

Our second specific objective is to use our model to analyze the trade-offs between yield in biomass, expected harvest rate, and the probability of catching a trophy-size fish when different fishing rates and size-limits regulations are applied, including minimum and maximum size limits and harvest slot size limits (Noble and Jones 1999). Based on these results, we discuss the general value of size regulations to accommodate take-fishing while maintaining the quality of fishing through the preservation of large fish.

Finally, our third objective is to evaluate the robustness of the conclusions derived from a per-recruit analysis to the existence of density dependence in the stock–recruitment relationship. Per-recruit analyses examine how growth, natural mortality, and fishing interact to determine the most appropriate fishing strategy, without considering the possibility of changes in recruitment (and reproductive capacity) due to change in stock size. Considering that for the majority of continental fisheries no time series of abundance data are available to allow estimation of stock–recruitment parameters (Pitcher and Hollingworth 2002; Radomski 2003), our underlying goal is to assess the ability of per-recruit analyses to capture essential features of trout recreational fishery management, without the need to explicitly consider the effects on recruitment.

To anchor our analysis on realistic biological and management reference points, we use as a case study the steelhead (sea-run rainbow trout, *Oncorhynchus mykiss*) fishery in the Santa Cruz River of southern Patagonia, Argentina (Pascual et al. 2001). Several characteristics of this population make it well suited for our analyses. Santa Cruz steelheads (SCS) are highly iteroparous anadromous trout, reaching large sizes through multiple yearly ocean migrations throughout their life cycle (Riva-Rossi et al. 2007). SCS undergo exposure to the fishery on entering the river to overwinter or spawn every year (Riva-Rossi et al. 2007). The SCS fishery is still developing, providing adequate estimates of run structure, size structure, and somatic growth for the unfished state. Because it is an exotic trout, uniquely found in this river, with a marine diet dominated by highly abundant zooplankton (Ciancio et al. 2008*a*), we expect density-dependent effects in the ocean to be weak and, therefore, growth to be largely invariant with density (an assumption of our model). The fishery is becoming a destination for national and international fly-fishers, with a growing provision of lodging and guiding services, and also accommodates more traditional local fishers that keep fish for consumption. Anglers are restricted to fish with a single hook, and there are daily and a seasonal creel limits of one and two individuals above 550 mm, respectively. Determining allowable rates of fishing mortality dominates the management agenda for this resource. The SCS fishery provides a good model for other sea-run trout fisheries in Atlantic river basins of southern Patagonia, such as sea trout (sea-run brown trout, *Salmo trutta*) in the Gallegos and Grande rivers (Ciancio et al. 2008*b*), with similar biological characteristics and management requirements.

Materials and methods

Our IBM has two main components. The first one, a von Bertalanffy growth model (VBGM) with individual var-

Table 1. Model parameters.

Parameter	Value
Finite rate of natural mortality	$A = 0.309$
River entry or vulnerability to fishery (%)	Age 3 = 40; age 4 = 50; age 5–13 = 100
Age-specific maturation (%)	Age 3 = 20; age 4 = 70; age 5–13 = 100
Parameters length–mass function	$a = 0.000007$ (g·mm^{-b}); $b = 3.0736$
Mean parameters for VBGM	$L_\infty = 750.69$ (mm); $k = 0.4893$ (year^{-1}); $t_0 = 1.1864$ (year)
Standard deviation and correlation of growth parameters	SD(l) = 65.24; SD(t) = 0.2867; cor(l,t) = 0.274
Residual variance in growth model	Var(ε) = 13.8943
Slope of linear relationship between natural mortality and size	$c = 0.18$ (mm^{-1})

Note: Rates are expressed as proportion or percentage calculated on an annual basis. VBGM, von Bertalanffy growth model.

iation, is used to simulate individual growth trajectories representing population-level variability in growth. Growth parameters of individual fish are random variables that deviate from the population means. The second component, an individual-based yield-per-recruit (YPR) model, represents the history of a cohort over time as individuals grow and experience natural mortality, maturation, river–ocean migrations, and in-river fishing mortality. Different size regulations and fishing rates are introduced as size-specific probabilities of individual fish being harvested in a particular year. The final outcomes of the model are the total number of fish surviving and total number harvested, together with their size and mass distributions. Spawning biomass is calculated as a function of maturation and individual mass.

Case study and basic data

The Santa Cruz is the second largest river of Argentinean Patagonia (50°S, 70°W; average discharge 690 m^3·s^{-1}; SSRH 2005). It flows for 382 km across the Patagonian plateau to drain into the Atlantic Ocean (Pascual et al. 2001).

The general life history plan of SCS has been described by Pascual et al. (2001), Riva-Rossi et al. (2003), and Riva-Rossi et al. (2007), based on samples of adults collected with gillnets and by anglers during the spawning migration (period 2000–2008) and from radiotelemetry studies. Recreational fishing takes place for the most part close to the estuary, around the town of Piedra Buena, when SCS enter the river from the ocean in March–April. Spawning occurs between August and October, primarily in locations along the mainstem river. Most individuals have a 2-year freshwater residence (few fish smolt at ages 1 or 3). Fish are fully vulnerable to the in-river fishery and mature at age 5, with partial vulnerability and maturation at ages 3 and 4. We used direct aging of fish caught in-river, supported by scale pattern analysis, to estimate that 40% of fish become vulnerable to the fishery at age 3 and 30% more at age 4 (Table 1). However, not all fish that enter the river mature; only 20% of vulnerable fish at age 3 mature, (i.e., 8% of age-3 fish), and at age 4, 70% of all vulnerable and immature fish mature (Table 1). The oldest fish registered was 11 years old and had spawned in 8 consecutive years. Finite rate of natural mortality (A) was estimated by catch curve analysis assuming that the current age structure is close to that of the virgin population (Table 1). For aging and growth analysis (next section), we used scales extracted from an area below the posterior margin of the dorsal fin and above the lateral line. Complementary, fish were measured (fork length, FL, mm), weighed (g), and sexed.

Analysis of growth

Individual growth trajectories were obtained through back-calculated size-at-age from scale measurements on fish with the predominant 2-year freshwater residency. Annual marks (annuli) were recognized using general criteria for temperate fish (Tesch 1971) and experience with SCS (Riva-Rossi et al. 2007). The radii corresponding to different annuli were measured from the nucleus and along the longitudinal anterior axis of the scale. Age and radii measurements were recorded for a total of 135 individual fish caught between May 2004 and November 2005. The biological intercept back-calculation method was employed to estimate lengths-at-age for each individual (Campana 1990):

$$(1) \qquad FL_i = FL_c + \frac{(r_i - r_c)}{(r_c - r_0)} \times (FL_c - FL_0)$$

where FL_i is the estimated fork length at age i, FL_c is the fork length at capture, r_i is the scale radius at age i, r_c is the scale radius at capture, and FL_0 and r_0 are the fork length and scale radius, respectively, at the start of proportionality between the somatic growth and the scale growth. We used 30 mm and 0.07168 mm as values of FL_0 and r_0, respectively. These values represent the mean fork length at scale formation and the mean scale radius measured from the focus to the first circulus, both obtained on hatchery fish (J. Lancelotti, CENPAT-CONICET, Puerto Madryn, Argentina, personal communication, 2005).

A VBGM curve (Quinn and Deriso 1999) was fitted to the individual profiles of marine growth (excluding freshwater ages 1 and 2) using nonlinear mixed-effects models (Davidian and Giltinan 1995; Pinheiro and Bates 2002) as previously done by Schaalje et al. (2002) and Escati-Peñaloza et al. (2010). Mixed-effects models facilitate the incorporation of different sources of growth variability by including "fixed effects", associated with the population means, and "random effects", associated with individual variability. Under this method, growth parameters of each individual fish are expressed as the sum of a population mean parameter (fixed effect) and an individual deviation (random effect) (Lindstrom and Bates 1990):

$$(2) \qquad L_{ij} = (L_\infty + l_i) \times \left\{1 - e^{-(K+k_i)\times[age_{ij} - (t_0 + t_i)]}\right\} + \varepsilon_{ij}$$

where L_{ij} represents length at age j for the ith fish; L_∞, K, and t_0 are the VBGM population mean parameters (fixed effects); and l_i, k_i, and t_i are individual deviations (random effects) from the VBGM population mean parameters for the ith fish, assumed to be normally distributed, with zero mean

and variance–covariance matrix Σ, a 3×3 matrix with diagonal elements var(l), var(k), and var(t_0) and off-diagonal elements given by cov(l,k), cov(l,t), and cov(k,t). The term ε_{ij} represents process stochasticity assumed to be normally distributed with zero mean and variance–covariance matrix $\sigma_\varepsilon^2 A$.

The Akaike information criterion (AIC) and the likelihood ratio test (LRT) (Burnham and Anderson 1998) were used to asses several nested VBGMs of varying statistical complexity in random effects. Parameters and their variance–covariance matrix were estimated using maximum likelihood methods implemented in the nlme library (Pinheiro et al. 2009) in R software (R Development Core Team 2009). Alternative mixed-effects models, based on the Richards, Logistic and the Gompertz growth models, were fitted to the individual length data, but the VBGM was favored by an AIC analysis.

Individual-based demographic model

We built an individual-based version of the generic age-structured YPR model (Quinn and Deriso 1999) and implemented it in Visual Basic for Applications (Microsoft Excel). In IBMs, each individual can be assigned different status or characteristics (age, sex, maturity, social status) and it is tracked through time (DeAngelis and Gross 1992; Grimm and Railsback 2005). In our case, the model incorporates individual variability in growth and the associated probability of harvest as a function of individual size.

A run of our model consisted of a Monte Carlo simulation of the history of a cohort of 300 000 fish through time, from age 3 (recruits, R) to age 13, under a given exploitation scenario (see below). Each of the 300 000 fish was assigned its own set of growth parameters, L_∞, K, and t_0, based on the mixed-effects model (previous section). These values were drawn from a multivariate normal distribution with mean vector μ (population mean parameter) and variance–covariance matrix Σ (for the "best model"; previous section). At each age or time step, each fish was exposed sequentially to five stochastic processes: growth, river entry and vulnerability to the fishery, maturation, angling, and natural mortality. Fish grew according to the VBGM (eq. 2), based on their individual set of parameters, plus a normal random residual (0, var(ε)). For each fish and remaining processes, a random uniform number (0, 1) was drawn to decide whether the event (i.e., vulnerability, maturation, catch, or natural death) occurred or not. For our base runs, we used age-dependent probabilities for vulnerability and maturation and an invariant probability of death by natural causes (Table 1).

For a given age, all vulnerable fish are equally likely to be caught (with probability equal to the fishing rate), but only those within the size limits are sacrificed, while the others are assumed to be released and to survive. Therefore, whereas fishing rate is age- and size-independent, realized catch is age-dependent through the vulnerability-at-age function (Table 1), and harvest (i.e., sacrificed fish) is size-dependent through the size-limit regulation. Several simulation experiments were conducted, each one consisting of a given management scenario of size limits and fishing rate (details below). Different indices of fishery performance and population status were calculated for each management scenario.

YPR is the total harvest (kg) per recruit, calculated as the sum of the individual masses of fish harvested over the 10 time steps divided by R. Individual masses are a power function of individual length (parameters in Table 1):

$$W = a \times L^b \quad (3)$$

Relative run size is the number of surviving fish entering the river summed over all ages relative to the corresponding number for an unfished stock. It provides an index of population–run size per recruit under different harvest strategies, assumed to be proportional to the expected catch in numbers per unit of effort by an average fisherman.

Relative average size of fish is the average size of all vulnerable surviving fish summed over all ages, relative to the corresponding value for the unfished stock. It provides an index of the average size of fish an angler can expect to catch.

Relative abundance of trophy-size fish is the number of vulnerable surviving fish summed over all ages, larger than a trophy size of 760 mm (90th percentile of virgin stock), computed relative to the corresponding number in the unfished stock. It provides a relative index of the probability for an angler to catch a trophy-size fish.

Relative spawning biomass (S/B_0) is the sum (over all ages) of the individual masses of all surviving mature fish, computed relative to the corresponding number in the unfished stock. It provides an index of eggs per recruit.

Relative recruitment (R/R_0) is the recruitment expected from the spawning biomass remaining after harvest, computed relative to its value for the virgin population and for different scenarios of population "productivity" (unknown for SCS). Relative recruitment (R/R_0) as a function of relative spawning biomass was modeled by a Beverton–Holt stock–recruitment relationship reparameterized in terms of a productivity parameter known as "steepness" (h), corresponding to the relative recruitment when S/B_0 is equal to 0.20 (Mace and Doonan 1988):

$$R/R_0 = \frac{S/B_0}{\alpha + \beta \times S/B_0} \quad (4)$$

$$\alpha = \left(\frac{1-h}{4 \times h}\right) \quad (5)$$

$$\beta = \frac{5 \times h - 1}{4 \times h} \quad (6)$$

Steepness values for salmonids were obtained from a meta-analysis of spawner–recruit time series for many species and families conducted by Myers et al. (1999). The estimated median value of steepness for salmonids was 0.85, with 20th and 80th percentiles of 0.8 and 0.89, respectively. We calculated relative recruitment based on these median and percentile values, in addition to lower, more conservative steepness values of 0.6 and 0.7.

The first index of fishery performance, YPR, is expressed in mass units (kg). Meanwhile, the remaining five indices are all dimensionless, expressed as relative values with respect to the corresponding value for the unfished population.

Management scenarios

We examined the performance of different combinations of size limits and fishing rates by conducting two sets of simula-

Table 2. Simulated management scenarios.

	Scenario	
Harvest control	1: Variable fishing rate	2: Variable size limit
Minimum size limit (mm)	550	300 to 900 (step 10)
Maximum size limit (mm)	900	300 to 900 (step 10)
Annual fishing rate	0.05 to 0.40 (step 0.05)	0.10

Note: Shaded cells represent management controls that stayed fixed, while unshaded cells represent variables that were changed across simulations. For Scenario 1 (variable fishing rate), the step between fishing rates was 0.05; a maximum size limit of 900 mm implies that the only effective restriction is in the minimum size limit. For Scenario 2 (variable size limits), the step between sizes was 10 mm, and all possible combinations of maximum and minimum size limits (each defining a slot) were evaluated (see text for details). Annual fishing rate is expressed as proportion captured per annum.

tions (Table 2). In the first set, referred to as "variable fishing rate", we simulated the current minimum size limit for SCS (550 mm) at different fishing rates (from 5% to 40% by 5% increments). In the second set, called "variable size limits", we kept fishing rate at 10% and changed the size limits using all combinations of minimum and maximum size-limit regulations within the range 300–900 mm, by steps of 10 mm. For practical purposes, and because virtually no fish grow larger than 900 mm, setting an upper size limit of 900 mm is equivalent to a minimum size regulation. Similarly, few vulnerable fish are smaller than 300 mm, and setting a lower size limit of 300 mm is equivalent to maximum-size regulations. Combinations with lower limits higher than 300 and upper limits lower than 900 provide harvest-slot regulations.

Generalization of results

Our base model includes individual variability only in growth, but assumes that other parameters, such as natural mortality, river entry, or maturation are independent of fish size. Whereas these parameters may be affected by size in fish (reviewed by Rose et al. 2001), we have no data to support any specific parameterization of such relationships. To explore the sensitivity of our results to departures from those basic assumptions, we performed an additional set of simulations, similar to those in the variable size limits (Table 2), but with natural mortality varying with size. We made natural mortality of individual fish a linearly decreasing function of the individual asymptotic size (i.e., larger fish have higher survival):

$$(7) \qquad A_i = A - c \times (L_{\infty,i} - L_{\infty})$$

where A, the mean natural mortality, is equal to that of our base case (30.9% per year; Table 1), L_{∞} is the mean parameter for the population, and $L_{\infty,i}$ is the corresponding parameter for the individual fish i, equal to $(L_{\infty} + l_i)$, and c was chosen to ensure a significant level of individual variation in natural mortality; the 5th and 95th percentiles of the individually assigned mortalities (A_i in eq. 7) were 12% and 50%, respectively (Table 1). This formulation of size-dependent natural mortality would represent better the apparent faster growth of older fish in our samples (Fig. 1).

Modifications in the mortality assumption affected the size structure of simulated populations. Because we defined trophy size as the 90th percentile of the size distribution of the unfished stock (760 mm in our base case), we recalculated the trophy size for the size-dependent mortality scenario to ensure consistency in results involving the corresponding fishery performance indices.

Results

IBM for somatic growth

The reconstructed trajectories of size-at-age show a significant individual variability in growth, whereas individual trajectories have similar shape (Fig. 1*a*). Our model captures the individual differences in growth as individual variation in the parameters of the VBGM. Convergence problems were encountered every time we tried to incorporate individual variability in the K parameter independently of the starting values (Table 3). The LRT and AIC values indicated that among the remaining models, model 3 (random effects on L_{∞} and t_0) had a significantly better fit than alternative, more restrictive formulations (Table 3, parameters in Table 1):

$$(8) \qquad L_{ij} = (L_{\infty} + l_i) \times \left\{1 - e^{-K \times [age_{ij} - (t_0 + t_i)]}\right\} + \varepsilon_{ij}$$

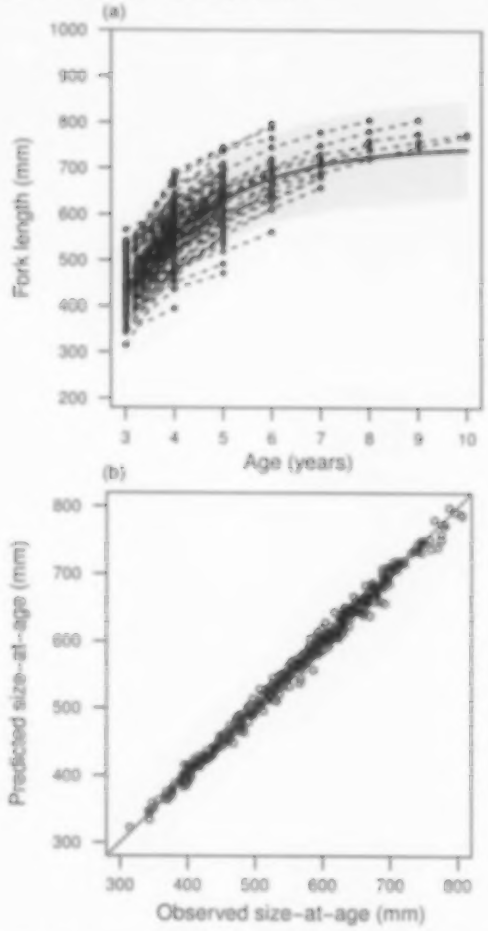
This model provided an adequate representation of the individual growth trajectories (Fig. 1*b*). The few old fish in our data set (six fish older than age 8), however, are larger at older ages than the average length predicted by the mixed-effects model (Fig. 1*a*), hinting at a possible negative relationship between size and mortality (see below).

Effects of increasing fishing rate on different fishery indicators

We explored the per-recruit effects of increasing the annual fishing rate while maintaining the current minimum size limit of 550 mm on relative run size, relative abundance of trophy-size fish, relative average fish size, and yield in mass (Figs. 2*a*–2*d*). Relative run size declined moderately with fishing rate (about 20% reduction for a 20% fishing rate; Fig. 2*a*), while the number of fish larger than 760 mm in the run declined sharply (about 60% reduction for a 20% fishing rate; Fig. 2*b*). The average fish size in the run was largely insensitive to fishing rate (Fig. 2*c*). In general, fishing rates of 10% had minimal effects on average size, moderate effects on run size, and strong effects on the abundance of trophy-size fish (reduction of 40%). Meanwhile, yield in biomass per recruit increased monotonically with fishing rate (Fig. 2*d*), exhibiting a clear trade-off with the other indicators of fishing quality.

Increasing fishing rates significantly reduced relative

Fig. 1. von Bertalanffy growth model fitted by treating individual differences as random effects. (*a*) Dashed lines are observed individual growth trajectories, the solid line is predicted mean size-at-age, and the shaded area represents the 5% and 95% quantiles calculated from simulated growth trajectories. (*b*) Scatterplot of observed size-at-age versus predicted size-at-age values.



spawning biomass (about 30% reduction for a 20% fishing rate; Fig. 2*e*). However, the expected reductions in relative recruitment resulting from these spawning levels and for typical steepness values reported for salmonids were marginal. Even for values of steepness that could be considered low for salmonids (h = 0.6), recruitment was not reduced by more than 20% under fishing rates as large as 40% per year. Therefore, most effects on fishery performance are to be expected from postrecruitment impacts of fishing. Hence, per-recruit analyses (e.g., Figs. 2*a*–2*d*) largely capture the effects to be expected from fishing, particularly for low fishing levels such as those that would be applicable if the recreational quality of the fishery, as expressed by availability of trophy-size fish, were to be preserved (<10% decline).

Size limits, trade-offs, and fishery performance

Considering the fast deterioration of fishing quality with fishing rate, we next explored the value of size-limit regulations for improving fishery performance. We applied a 10% fishing rate to various retention size slots and looked at changes in relative run size, relative abundance of trophy-size fish, and YPR (Fig. 3). All three indices varied markedly with changing size limits. In general, and as expected, restricting slot sizes resulted in increased run size and abundance of trophy-size fish, with reductions in yield. However, the different shapes of the response surfaces suggest that given the unavoidable trade-off between fish removal and preservation of large fish in the population, there may be room for improvement in overall performance relative to the current regulation.

Table 3. Results of model selection.

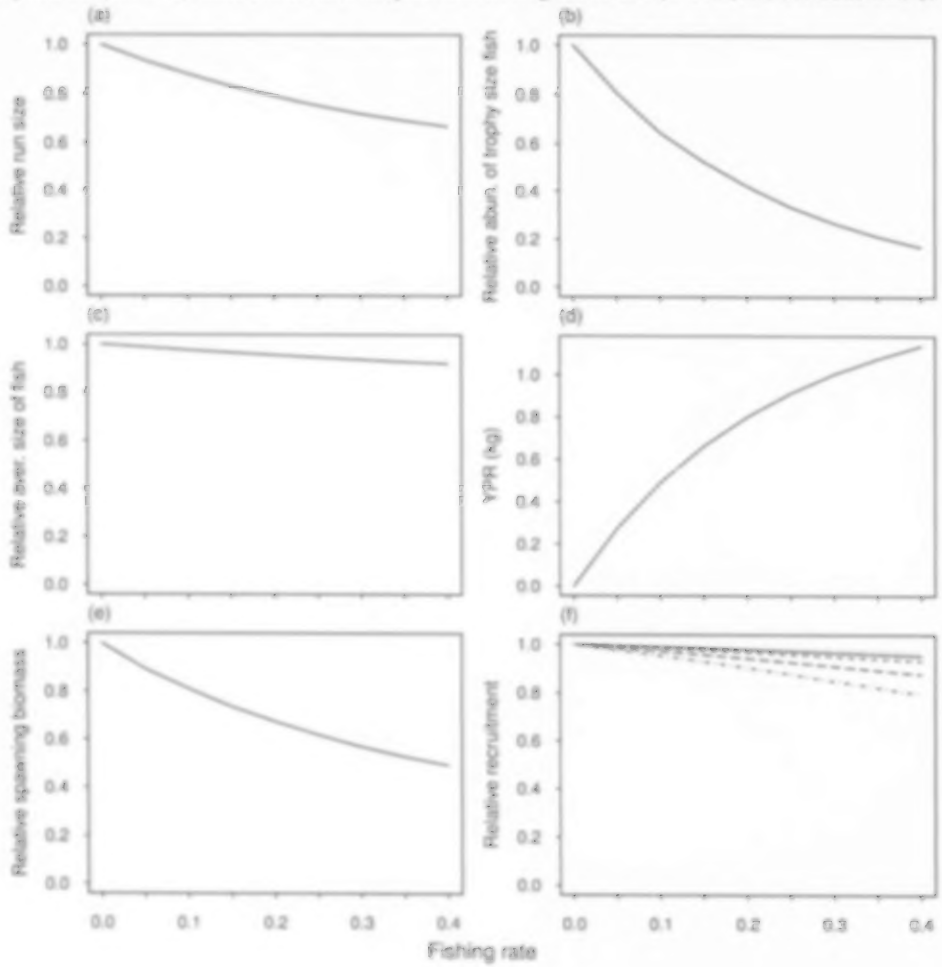
Model		Fit and selection		
No.	Random effects	LL	LRT	AIC
1	L_∞, K, t_0	NC	NC	NC
2	L_∞, K	NC	NC	NC
3	L_∞, t_0	−1850.733	—	3715.466
4	L_∞	−1900.391	3 vs. 4***	3810.781
5	t_0	−1944.494	3 vs. 5***	3898.988
6	K, t_0	NC	NC	NC
7	K	NC	NC	NC

Note: LL, log-likelihood; LRT, likelihood ratio test; AIC, Akaike's information criterion; NC, no convergence; ***, $p < 0.0001$ (3 vs. 4: χ^2 = 99.3149, df = 2; 3 vs. 5: χ^2 = 187.5214, df = 2).

Trade-offs are better explored by plotting two value measures against one another (Walters and Martell 2004). Overall, the trade-offs between run size and yield and between abundance of trophy-size fish and yield have a similar shape (Fig. 4). The current minimum size limit of 550 mm provides near-to-maximum yield but, being very liberal and providing very little size selectivity in harvest, results in substantial reductions in run size and abundance of trophy-size fish. Both quality indicators could be considerably enhanced by setting more stringent size limits. Meanwhile, alternative size limits perform very differently under the two trade-offs considered. In the trade-off between run size and yield, minimum size-limit regulations lie along a Pareto Frontier (Walters 1986; Walters and Martell 2004) — a convex trade-off curve at the outer boundary of feasible solutions where no further improvement can be made on one axis without negatively impacting the other (Fig. 4*a*). Harvest strategies on this boundary provide better solutions to the trade-off than any other alternative strategy. Maximum size regulations, on the other hand, provide the poorest solutions to this trade-off, and slot sizes have intermediate performance. The relative performance of minimum and maximum size limits is inverted when the trade-off between the abundance of trophy-size fish and yield is considered (Fig. 4*b*). Although some slot regulations perform better than maximum size limits, the improvement is marginal. Maximum size limits provide close-to-best solutions for this trade-off. Minimum size regulations, on the other hand, provide the poorest performance.

Overall, these results indicate that for a given yield level, minimum size regulations are best at maintaining large runs and maximum size regulations are best at preserving large fish. Because no harvesting strategy simultaneously provides optimal solutions to the trade-offs between yield and run size and between yield and abundance of trophy-size fish, man-

Fig. 2. Effects of increasing fishing rate on different performance indicators for a minimum size limit of 550 mm: (*a*) relative run size, (*b*) relative abundance of trophy-size fish, (*c*) relative average size of fish, (*d*) yield-per-recruit (YPR), (*e*) relative spawning biomass, and (*f*) relative recruitment (steepness for salmonids from Myers et al. (1999): solid line median ($h = 0.85$), short dashed line 20th percentile ($h = 0.8$), dotted line 80th percentile ($h = 0.89$); more conservative steepness values: long dashed line ($h = 0.7$) and dot-dashed line ($h = 0.6$)).

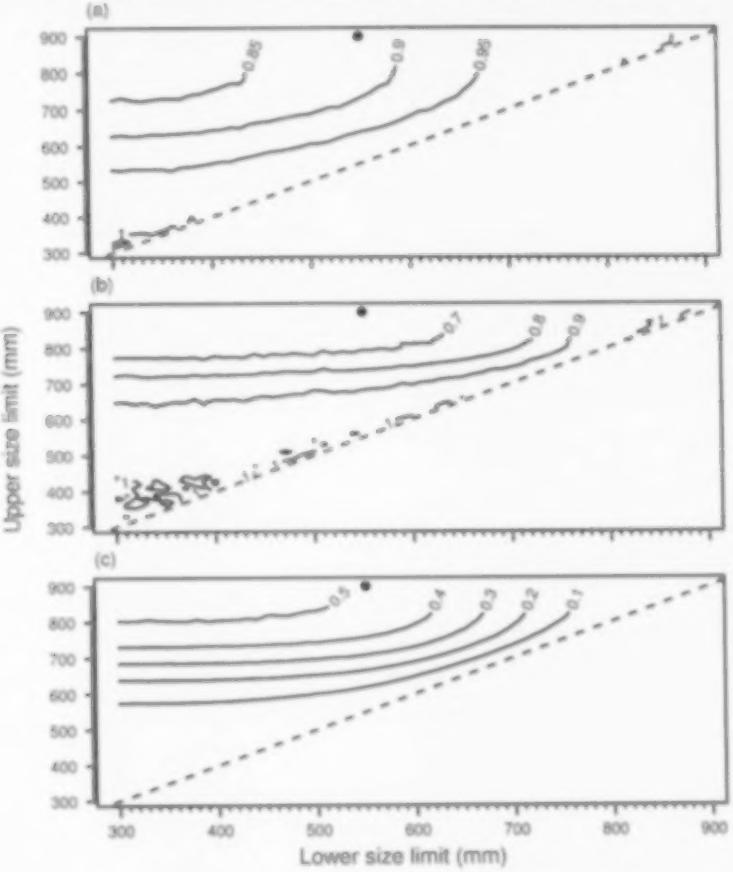


agement options need to be considered by conditioning on two of the indicators and finding the optimum with respect to the third indicator. For instance, if fishing quality is favored over yield, we may select the strategy that maximizes yield among the subset of strategies that keep run size and the abundance of trophy-size fish above a certain minimum acceptable level. If, for example, the goal is to keep run size and abundance of trophy-size fish at 90% of virgin values, a subset of size limit options can be identified (shaded area in Fig. 4). Within that subset, a maximum size limit of 630 mm maximizes yield. Whereas keeping run size at 90% of virgin values would allow for higher yields (as large as 0.448 kg per recruit), keeping the abundance of trophy-size fish at 90% of virgin values imposes a more stringent constraint, limiting maximum yield to 0.263 kg per recruit. An overall optimum, given the constraints, is obtained with a slot size of 500–680 mm (Fig. 4), but the improvement in yield with respect to a maximum size limit of 630 mm is not large (0.077 kg).

Size-dependent natural mortality

The trade-offs between fishery quality indicators were not substantially modified when natural mortality was assumed to decrease with size (Fig. 5). It must be understood that the change in the mortality assumption modified not only the size structure of the unfished stock, but also its size in total number of fish. Absolute run size, YPR, and numbers of trophy-size fish, therefore, varied accordingly. However, by expressing fishery performance indices of run size and trophy-size fish as relative values with respect to those of the unfished stock, we were able to look at the general shape of the trade-offs. Results indicate that whereas the specific values of YPR emerging from different size regulations change somewhat with respect to those in the base case

Fig. 3. Performance of various size-limit regulations under 10% fishing rate. Shown are isopleths for three indicators: (*a*) relative run size, (*b*) relative abundance of trophy-size fish, and (*c*) yield-per-recruit (kg). Relative run size and relative abundance of trophy-size fish are in relation to the virgin stock. Solid circle (●) represents a minimum size limit of 550 mm (current regulation for SCS).



(Fig. 4), the main features of the trade-offs were kept. Namely, the shape of the trade-offs is virtually the same and the relative impact of the current size regulations, as well as minimum and maximum size-limits regulations on the three fishery indicators, remain unchanged.

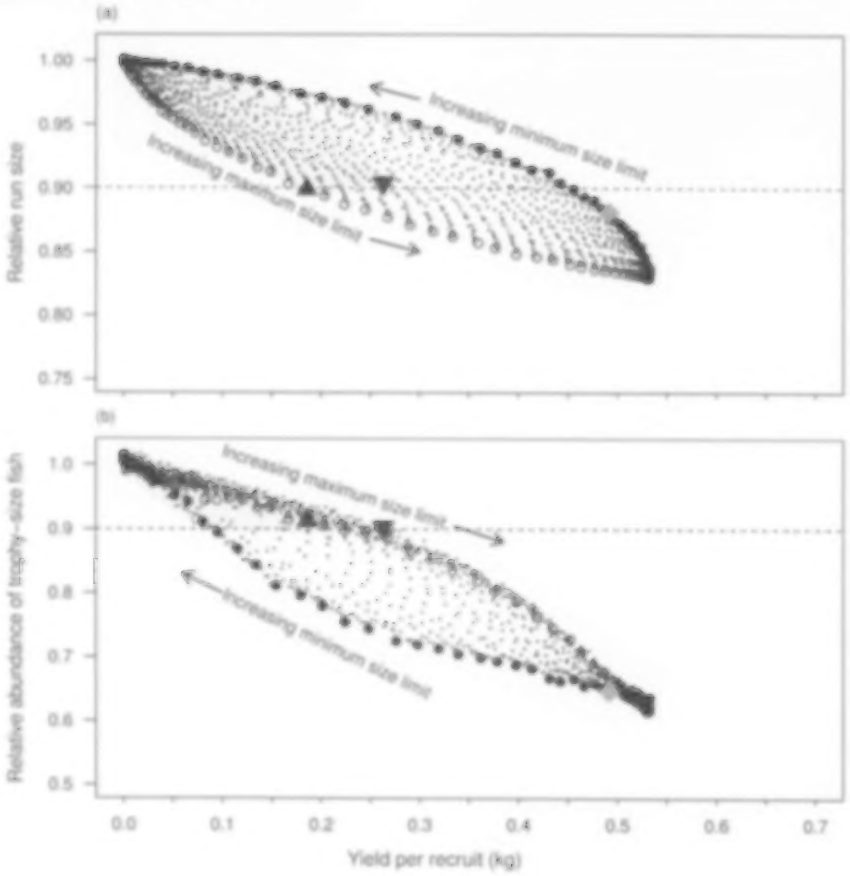
Discussion

We developed a general framework to analyze the value of alternative management strategies in recreational fisheries, which captured the complexities associated with multiple objectives and to individual-based population demographic processes.

To measure performance we used a suite of indicators of population state and fishing quality that capture essential drivers of anglers' satisfaction. Arlinghaus (2006) found that German anglers attach relatively little importance to catch motives during interviews; however, their realized satisfaction with the fishing experience is still mainly catch-dependent. We believe this is true for many trout and other game fisheries around the world. In such fisheries, catch-related, activity-specific components of anglers' satisfaction (those related to the fishing activity itself as compared with those common to all outdoor recreation activities), such as the number of large fish, the number of consumable fish, or the quantity of fish bites, comprise a most important part of overall satisfaction (Arlinghaus 2006). These catch-dependent components are summed up by three fishing quality indicators, namely YPR, run size, and the availability of trophy-size fish.

In a classic paper, Jensen (1981) analyzed the harvest of large or "trophy fish" based on an average-growth in a YPR model. Harvest in numbers of old fish (those ages with average length above some defined trophy size) was used as the response variable, and harvest rate and age of first capture were the controls. But because size in fish is not only associated with age but with individual growth rates, the abundance of large fish is only loosely correlated with the abundance of old fish. In addition, average-growth models cannot capture the compounded effect on size structure of selectively removing fish with different growth rates (Sainsbury 1980; Parma and Deriso 1990) by applying different size regulations. Combining mixed-effects growth models and individual-based harvest models provides a full description of the relationship of growth, mortality, and size structure (including the provision of trophy-size fish), as well as a general frame-

Fig. 4. Performance of various size-limit regulations under 10% fishing rate when natural mortality is constant: trade-offs between (*a*) yield-per-recruit and relative run size and (*b*) yield-per-recruit and relative abundance of trophy-size fish. Each point corresponds to a specific size-limit regulation, identified by the following symbols: (●) minimum size limit, (○) maximum size limit, (+) harvest slot size limits, (grey +) minimum size limit of 550 mm (current regulation for SCS), (▲) maximum size limit that maximizes yield (630 mm), and (▼) harvest slot size limits that maximizes yield (500–680 mm). Shaded area identifies the subset of size limits that maintains run size and abundance of trophy-size fish within 90% of their virgin values.



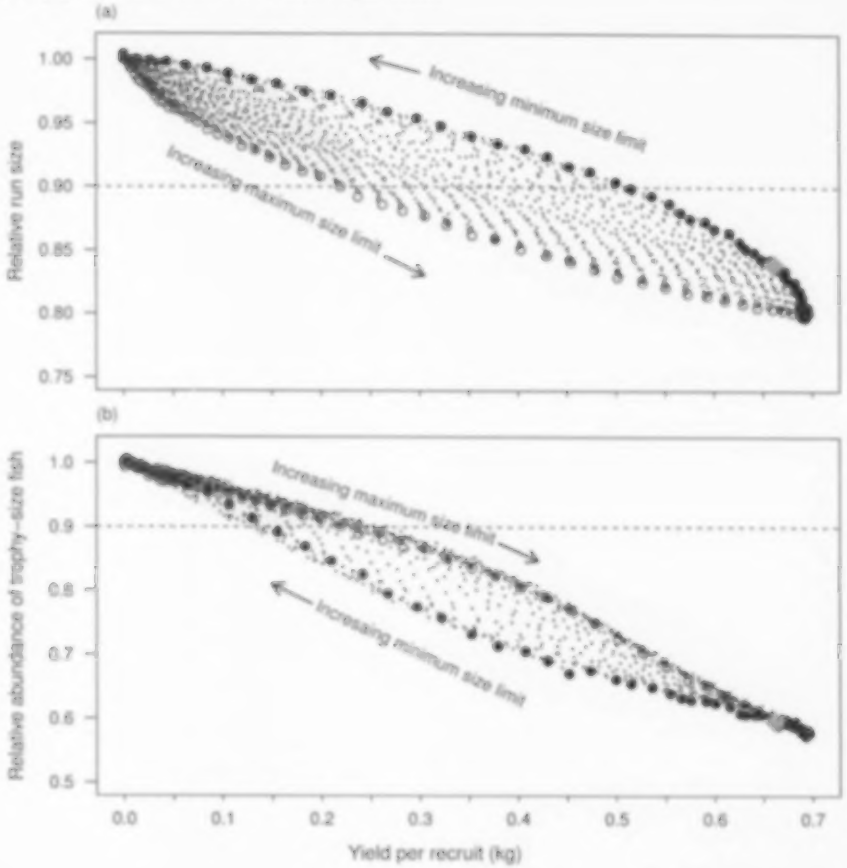
work for policy analysis of size-based harvest strategies and objectives in fisheries.

By applying this framework to a case study, we were able to derive some general principles to support the management of recreational fisheries. Moderate levels of harvest produced negligible changes in average size, total abundance, and even recruitment rate, but resulted in important reductions in the abundance of trophy-size fish. In developing recreational fisheries, anglers and managers are likely to notice a decline in catch rates of trophy-size fish before declining run size or overall catch rates. This is consistent with our experience in Patagonia, where anglers report the reduction in size as a primary problem and the reduction in numbers as less of a concern (Pascual et al. 2002; Casalinuovo et al. 2002; Vigliano et al. 2008). It is also consistent with previous work where no apparent changes in total densities of fishes were detected between fished and unfished sections of Spanish rivers that showed sharp differences in age structure and life span (Braña et al. 1992).

The recognition in recent years of the potential of recreational fishing to adversely impact fish populations has led many authors to emphasize the need to limit fishing effort (Post et al. 2002; Walters and Martell 2004). The fact that trophy fishing is so sensitive to harvest indicates that keeping large fish may only be possible through very low take levels. Whereas controlling fishing effort appears as the most direct way to reduce fishing mortality, catch-and-release practices provide an alternative. It must be understood, however, that hooking mortalities incurred under strict catch-and-release policies may not be insignificant when fishing pressure is high (up to 10%, from a meta-analysis conducted by Bartholomew and Bohnsack 2005). In intensively exploited recreational fisheries, the effectiveness of catch-and-release strategies is inevitably linked to low levels of postrelease mortality (Coggins et al. 2007; Pine et al. 2008).

As traditional tools to manage recreational fisheries have proven unable to limit fishing effort, the addition of fish has been regarded as a possible tool to compensate for the effects

Fig. 5. Performance of various size-limit regulations under 10% fishing rate when natural mortality is size-dependent: trade-offs between (*a*) yield-per-recruit and relative run size and (*b*) yield-per-recruit and relative abundance of trophy-size fish. Each point corresponds to a specific size-limit regulation, identified by the following symbols: (●) minimum size limit, (○) maximum size limit, (·) harvest slot size limits, (grey ○) minimum size limit of 550 mm (current regulation for SCS). Shaded area identifies the subset of size limits that maintains run size and abundance of trophy-size fish within 90% of their virgin values.



of fishing. Stocking is one of the most common practices, if not the most common, used in inland fisheries management today (Cowx 1994; Welcomme 2001). However, most stocking programs are conducted without a clear definition of objectives or without a proper assessment of potential or actual success (Cowx 1994). Our results show that large declines in fishing quality can be expected at fishing mortalities that are much too low to cause a sizeable decline in recruitment from virgin values. Thus, quality overfishing in these fisheries, a problem closely related to growth overfishing, could occur even with healthy levels of recruitment. In such cases, stocking of young fish at stages previous to those at which population bottlenecks operate would fail to produce any major improvement of fishing quality. Stocking could even have negative population-level impacts if somatic growth or mortality at juvenile stages were density-dependent (Ali et al. 2003).

Trade-offs among management objectives are particularly important in recreational trophy fisheries for trout. When somatic growth is density-independent, fish harvest occurs at the expense of other indicators associated with the quality of fishing experienced by individual anglers (e.g., trophy-size fish, number of bites); best fishing occurs at virgin stock levels. Harvest and fishing quality are conflicting objectives.

Meanwhile, our results illuminate some general management approaches to deal with conflicting objectives. Size limits provide a tool to better accommodate harvest without compromising fishing quality. For a given yield, minimum size regulations are best at maintaining larger runs, and maximum size limits are best at preserving large fish. Harvest slots provide some marginal improvements over maximum size limits to preserve large fish. However, slots are more difficult to assimilate by anglers and also more difficult to implement (Pierce and Tomcko 1998). For our case study, and we suspect that for recreational trout fisheries in general, the difficulties of implementing slot limits, together with their small marginal gain over maximum size limits, may lead managers to discard their use. In summary, the relative value of minimum or maximum size limits in trophy-size fisheries will depend on whether management is targeted towards preserving run-size or trophy-size fish. When preserving run size is favored over preserving trophy-size fish, minimum size limits constitute the best policy overall, whereas maxi-

mum size limits are best when the emphasis is on preserving trophy-size fish.

The balance among complexity, data availability, and generality needs to be considered when using models in general (Levins 1966) and IBMs in particular. IBMs provide a highly flexible tool for simulating individuals and populations, incorporating aspects usually ignored in analytical models (e.g., variability among individuals), and have the advantage that they are closer in details to real systems (Grimm and Railsback 2005). In fact, there is virtually no limit to the degree of complexities in relation to individual responses that they could accommodate. But this great flexibility comes at a cost; they can easily become too complex and difficult to analyze and understand (Grimm 1999; Grimm and Railsback 2005), and they can hide general, important patterns and loose generality (Levin and Pacala 1997). We kept the complexity of our model within the limits imposed by data availability. Nevertheless, it is pertinent to analyze how different simplifying assumptions in our model may compromise the robustness and generality of our results.

Our base model includes individual variation in growth, but does not consider concomitant variations in natural mortality or maturation. Our second round of simulations had natural mortality modeled as a function of each individual's maximum size. This simulation exercise shows that our general results regarding the shape of the trade-offs and the relative value of alternative management strategies for given management goals are quite robust to violation of the assumption of size-invariant mortality. Maturation, on the other hand, does not affect per-recruit fishery performance indices; therefore, most of our results are independent of the assumptions about the relationship between body size and maturation.

Another assumption in our model is that growth of adult, vulnerable fish is density-independent. This is a reasonable assumption in our case, but may not be so for populations where adults are affected by crowding (Rose et al. 2001). The extent to which density dependence could lessen the direct impact of the harvest on population size structure is of course case-specific. While consideration of density-dependent growth is beyond the scope of this paper, it would be straightforward to conduct such an analysis with our IBM approach.

From a methodological viewpoint, our results indicate that if preserving the abundance of trophy-size fish is a determinant goal, per-recruit analysis may capture the essence of the harvest problem without the need to consider stock–recruitment processes. This is because harvest rate must be kept at values that are low relative to those that are expected to impact recruitment. Long time series of population abundance and recruitment are rarely available for recreational fisheries (Pitcher and Hollingworth 2002; Radomski 2003); thus, it is encouraging that management guidelines can be derived from per-recruit analyses based solely on life history parameters.

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Seasonal changes in depth distribution of salmon sharks (*Lamna ditropis*) in Alaskan waters: implications for foraging ecology

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Abstract: The salmon shark, *Lamna ditropis*, is an endothermic, apex predator in the productive waters of the Gulf of Alaska (GOA). To understand their role in these productive habitats and to identify and better understand their interactions with commercially important species and fisheries, detailed information regarding the distribution of the salmon shark is required. Archival data records from eight recovered pop-up archival transmitting (PAT) tags were analyzed to examine the geographic and vertical distribution of female salmon sharks and elucidate how their distribution changes in relation to the biotic and abiotic environment. Tagged sharks used the neritic habitats of the GOA for the duration of the PAT deployments. Sharks exhibited a diel periodicity in their dive behavior. Salmon sharks exhibited three different patterns of depth distribution (behavioral modes) that occurred during distinct oceanographic seasons, likely reflecting changes in their foraging ecology in response to seasonal changes in the distribution and availability of important prey. The distribution of salmon sharks in the GOA appears to follow consistent patterns and has a high degree of geographical and vertical overlap with commercially important prey species. This information increases our understanding of interactions between salmon sharks and commercial fisheries and may assist in fostering responsible management for this species.

Résumé : Les taupes du Pacifique, *Lamna ditropis*, sont des requins endothermes, prédateurs de niveau trophique supérieur, dans les eaux productives du golfe de l'Alaska (GOA). Il est nécessaire d'obtenir des informations précises sur la répartition des taupes du Pacifique afin de comprendre leur rôle dans ces habitats productifs et d'identifier et de mieux évaluer leurs interactions avec les espèces et les pêches d'importance commerciale. Nous avons analysé les données archivées dans huit étiquettes émettrices détachables (PAT) récupérées pour étudier les répartitions géographique et verticale de taupes du Pacifique femelles et élucider comment leur répartition change en relation avec l'environnement biotique et abiotique. Les requins marqués ont utilisé les habitats néritiques du GOA pendant la durée de fonctionnement des PAT. Les requins possèdent une périodicité journalière dans leur comportement de plongée. Les taupes du Pacifique montrent trois patrons différents de répartition en profondeur (modes comportementaux) qui se manifestent durant des saisons océanographiques différentes, ce qui reflète vraisemblablement leur écologie de recherche de nourriture en réaction aux changements saisonniers dans la répartition et la disponibilité des proies importantes. La répartition des taupes du Pacifique dans le GOA semble suivre des patrons caractérisés et montre un fort degré de chevauchement géographique et vertical avec les espèces de proies d'importance commerciale. Ces informations augmentent notre compréhension des interactions entre les taupes du Pacifique et les pêches commerciales et peuvent aider à mettre en œuvre une gestion responsable de cette espèce.

[Traduit par la Rédaction]

Introduction

The spatiotemporal patterns of distribution of a species are fundamental to its ecology and population dynamics (Dingle and Holyoak 2001; Morales and Ellner 2002). The salmon shark (*Lamna ditropis*) (Hubbs and Follett 1947) is a wide-ranging apex predator (Nagasawa 1998; Brodeur et al. 1999) that uses neritic and oceanic habitats throughout the North Pacific Ocean (Nagasawa 1998; Hulbert et al. 2005; Weng et al. 2005). Salmon sharks have a broad depth distribution, with new electronic tag data showing them diving to depths of up to 1864 m (A.B. Carlisle and B.A. Block, unpublished data), and thermal range, occurring at temperatures from 2 to 24 °C (Nakano and Nagasawa 1996; Nagasawa 1998; Weng et al. 2005). As an abundant, active apex predator, the salmon shark may well play an important role in North Pacific ecosystems through top-down processes (Fogarty and Murawski 1998; Pace et al. 1999; Bascompte et al. 2005). As a result, an understanding of the spatiotemporal patterns of distribution of salmon sharks is an important part of understanding their ecological role (Kareiva et al. 1990; Crist et al. 1992; Morales and Ellner 2002) and is necessary for effective

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management of this species and the ecosystems of which it is a part (Block et al. 2002; Shillinger et al. 2008).

The broad geographic distribution of salmon sharks, particularly their use of cold, productive boreal waters, is likely associated with their endothermic capacity (Bernal et al. 2005; Weng et al. 2005). All sharks in the family Lamnidae have some degree of anatomical and physiological specialization associated with a capacity for heat conservation and endothermy, but the expression of these traits is considered greatest in the genus *Lamna* (salmon shark and porbeagle shark (*Lamna nasus*)) (Carey et al. 1985). Salmon sharks possess counter-current heat exchangers (retia mirabilia) that allow them to retain the metabolic heat generated by highly aerobic tissues in the muscles, viscera, kidney, and brain (Burne 1924). This enables the sharks to maintain body temperatures that are elevated by as much as 21.2 °C above ambient water temperature (Goldman et al. 2004). In addition, salmon shark cardiac physiology is also specialized for utilizing cold habitats. Through the increased expression of SERCA2 protein in the sarcoplasmic reticulum of cardiac myocytes, salmon shark hearts are able to maintain function at low temperatures, thereby providing their active, warm tissues with enough oxygen to effectively forage in cold environments (Landeira-Fernandez et al. 2003; Weng et al. 2005). These characteristics allow *Lamna* to be highly active predators across a large geographic and depth range and likely underlie their niche expansion into the productive boreal waters of the North Pacific (Block and Finnerty 1994; Goldman et al. 2004; Weng et al. 2005).

The highly productive neritic waters of the Gulf of Alaska (GOA) support some of the largest fisheries in the world (Mundy 2005), yet much remains unknown about the role of salmon sharks in this ecosystem or how individuals use this region. Although salmon sharks are opportunistic foragers with a diverse diet (e.g., Nagasawa 1998; Hulbert et al. 2005; Goldman and Musick 2008), they are known to feed on commercially important species, including Pacific salmon (*Oncorhynchus* spp.) and walleye pollock (*Theragra chalcogramma*) (Nagasawa 1998; Goldman and Musick 2008). Predation by salmon sharks may be an important component of natural mortality for these species, especially considering the abundance and high energetic requirements of salmon sharks due to their high metabolic rate (Nagasawa 1998; Bernal et al. 2005; Weng et al. 2008).

Salmon sharks exhibit life history characteristics typical of elasmobranchs (i.e., slow growth rate, late age at first maturity, and low fecundity) (Goldman and Musick 2006); therefore, they are particularly vulnerable to overexploitation (Musick et al. 2000; Cortes 2002) and need to be managed accordingly. While there is a small directed fishery for them in Japan (Goldman and Musick 2008), there is no commercial fishery for salmon sharks in US waters. However, there is a limited sport fishery that is primarily active in Prince William Sound (PWS), Alaska (Tribuzio et al. 2008). After the 1992 ban on pelagic drift gillnetting in the North Pacific (McKinnell and Seki 1998), much of the fishing mortality, especially in the form of nontarget catch, on salmon sharks in the eastern North Pacific shifted to neritic habitats. Nontarget catch of sharks in commercial fisheries in Alaskan waters is likely high but poorly documented (Camhi 1999) and may be a source of considerable mortality for salmon sharks, especially in the GOA and PWS (Goldman and Musick 2008).

Weng et al. (2005, 2008) and Hulbert et al. (2005) described general patterns of movement and vertical distribution of salmon sharks throughout their range in the eastern North Pacific. However, detailed analyses regarding changes in vertical distribution of salmon sharks in Alaskan waters, over both short (diel) and longer (seasonal) temporal scales, have not been conducted. We use high-resolution records from recovered PAT tags to address this lack of information and examine how salmon sharks use the neritic habitats of the GOA. Specifically, we describe seasonal changes in the geographic distribution and depth distribution of salmon sharks and associate these changes with those that occur in the surrounding environments. Detailed information regarding how depth distribution changes over time in relation to changes in the biotic and abiotic environment provides important insight into the ecological role of salmon sharks and their potential interactions with commercially important species and fisheries in the GOA.

Materials and methods

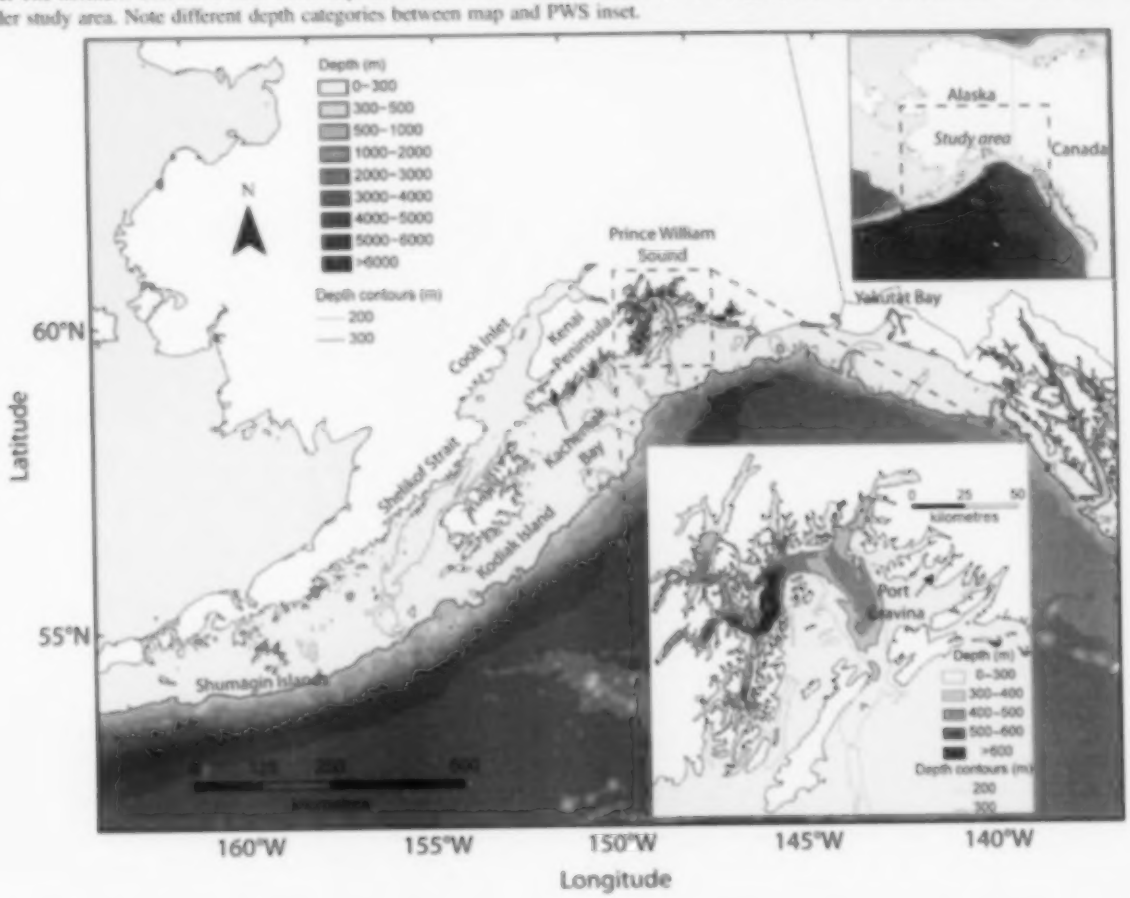
Electronic tagging

In July and August of 2002 to 2008, female salmon sharks were caught and tagged with electronic tags in Port Gravina, PWS, Alaska (60.75°N, 146.16°W; Fig. 1), by the Census of Marine Life Tagging of Pacific Predators program (TOPP) using the techniques described in Weng et al. (2005). Sharks were tagged with a Smart Position or Temperature Transmitting Tag (SPOT) (Wildlife Computers, Redmond, Washington), a PAT tag (Wildlife Computers, Redmond, Washington), or both (double-tagged). SPOT tags use the Argos system to provide geographic positions with a high degree of resolution when the tag is not submerged. Accuracy, or location class (LC), of positions is categorized by decreasing quality from LCs 3, 2, 1, 0, A, B, to Z. LC 3 positions have the best resolution (<150 m), followed by LC 2 (150–300 m) and LC 1 (350–1000 m), as determined by the Argos system (www.argos-system.org). Resolution of Argos LCs 0, A, and B have been estimated at 8 to 50 km (Hays et al. 2001; White and Sjoberg 2002), and LC Z does not provide a position.

PAT tags archive environmental data (temperature, pressure, light) at a user-designated interval and are programmed to detach from the study animal and float to the surface. Once at the surface, PAT tags transmit summaries of the archived environmental data to the Argos system. These summaries are used to reconstruct the movements and environment that the tagged animal experienced, even if the tag is not recovered (Block et al. 1998; Arnold and Dewar 2001; Gunn and Block 2001). However, physical retrieval of the PAT tag provides the entire archived time series for study, allowing for more detailed analyses to be performed (Teo et al. 2007; Weng et al. 2007).

Eight PAT tags with a mean deployment length of 121.9 ± 82.1 days (mean ± standard deviation (SD)) have been recovered from salmon sharks that used the northern GOA, giving access to a total of 1126 days of archival data. These sharks had a mean precaudal length (PCL) of 203.9 ± 18.3 cm, and all were assumed to be mature or large subadults based on

Fig. 1. The northern Gulf of Alaska. Inset maps show Prince William Sound (PWS), with tagging location (Port Gravina) indicated, and the broader study area. Note different depth categories between map and PWS inset.



size at maturity estimates of 164.7 cm PCL (Goldman and Musick 2006). All eight of the sharks were double-tagged with SPOT and PAT tags, providing the higher accuracy positions from SPOT tags to accompany the PAT records. Five of the recovered tags popped off on schedule and comprised the bulk of the data set (mean deployment, 205 ± 62.2 days; total data set, 1025 days) (Table 1). The other three tags were at liberty for approximately 1 to 1.5 months. Two of these sharks (nos. 119 and 097) were caught by sport fishermen. The third (no. 110) died for unknown reasons but did not exhibit abnormal behavior prior to its death and so was included in the study (Table 1). The PAT tag on shark 558 popped off ~1000 km west of Vancouver Island (45.79°N, 142.48°W) on schedule after 270 days, but we only analyzed the period of time when the shark was on the Alaskan shelf (the first 142 days). The final 23 days of 273 days of data from the PAT tag on shark 087 were corrupted, so only the first 250 days were analyzed. After removing these days from the analysis, there was a total of 975 days of data.

The PAT tags primarily recorded information during the summer, autumn, and winter. The longest PAT deployment (shark 087) lasted from the summer to the following spring (273 days) and was the only tag at liberty during the spring. During the first 3 days following tagging, shark 087 exhibited possible postrelease behavioral modification following tagging, and these days were not included in analyses (Hoolihan et al. 2011). Unusual behaviors were not apparent in the other sharks following tagging.

Data analysis

Movements were examined using Argos positions from the SPOT tags and geolocation positions from the recovered PAT tags. Argos positions from SPOT tags were filtered using a speed filter (1.5 $m \cdot s^{-1}$) to remove positions that represented biologically unrealistic rates of movement (Weng et al. 2005); all positions that fell on land or of LCs A, B, or Z were removed. Positions were then summarized to one position per day; where there were multiple positions in a day, the mean position was used.

Light level data from the PAT tags were used to generate daily estimates of longitude (Hill and Braun 2001), implemented with software provided by the manufacturer (WC-TSP version 1.02.008). Daily longitude estimates and sea surface temperature (SST) data recorded by the tags were matched with remotely sensed SST measurements to estimate latitude using the method described by Teo et al. (2004). To assess the accuracy of our geolocation estimates, for each day with an Argos position, the Argos position (or "true" posi-

Table 1. Female salmon sharks (*Lamna ditropis*) tagged in Port Gravina, Prince William Sound, with archival PAT records from the neritic waters of Alaska.

Shark no.	PCL (cm)	Tag types	Sampling interval (s)	Tagging date	Pop-up date	Pop-up location	Record length (days)	Behavior days	No. of days with Argos	Distance (km)
931	208	PAT/SPOT1	120	15 July 2002	14 Nov. 2002	54.96°N, 160.51°W	122	51, 63, 8	32 (26%)	1057
110	218	PAT/SPOT3	60	11 July 2004	8 Aug. 2004	60.69°N, 146.44°W	28[a]	28, 0, 0	3 (11%)	16
119	214	PAT/SPOT3	60	12 July 2004	8 Aug. 2004	60.34°N, 146.72°W	28[b]	27, 0, 0	1 (4%)	50
111	224	PAT/SPOT3	60	13 July 2004	9 Jan. 2005	59.29°N, 147.11°W	180	71, 69, 40	4 (2%)	164
097	207	PAT/SPOT3	60	13 July 2004	28 Aug. 2004	60.72°N, 146.11°W	46[b]	46, 0, 0	1 (2%)	4
087	206	PAT/SPOT3	60	22 Aug. 2005	22 May 2006	60.71°N, 147.53°W	250	65, 81, 104	15 (5%)	71
558	186	MK10/SPOT5	30	20 Aug. 2006	16 May 2007	45.79°N, 142.48°W	142[c]	28, 99, 15	51 (36%)[d]	1674
571	168	MK10/SPOT5	5	23 Aug. 2007	19 Feb. 2008	59.71°N, 139.52°W	180	51, 75, 54	34 (19%)	388

Notes: PCL, precaudal length; sampling interval, sampling interval of PAT tag; behavior days, numbers of days of the archival records designated as the different behavioral modes (B1, B2, B3); number of days with Argos, the number of days (percentage in parentheses) in the archival record with an associated high-resolution Argos position; distance, the distance between tagging location and pop-up or recapture location.

[a]Shark found dead on beach.

[b]Shark caught by sport fishermen.

[c]Record length is 270 days, but early analyzed data for period of time when shark was in neritic region of Alaska (first 142 days, to 8 January 2007).

[d]Refers to period analyzed in this study.

tion) was compared with the light-based estimate of longitude and the SST-based estimate of latitude. The errors in degrees, kilometres (great circle distance), and root mean square error (RMSE) (Teo et al. 2004) were calculated for latitude and longitude for each day when there was an Argos and geolocated position (Table 2). Based on 116 comparisons between Argos positions and geolocation estimates, we found that longitude geolocation estimates had a much lower error than estimates of latitude. The error in longitude was similar to published longitude error estimates from the northern GOA (41.7 km for tags moored at a depth of ~30 m, 123.5 km at a depth of 146 m) (Seitz et al. 2006). Because SST-based latitude estimates had a high level of error and Alaska has a longitudinally oriented coastline, we used longitude to estimate where the sharks were during the gaps of the Argos data.

Spectral analysis was used to examine the depth time series for periodic patterns. A fast Fourier transformation (FFT) with Hamming window smoothing (implemented using Matlab R2009b, The Mathworks Inc., Natick, Massachusetts) was applied to the full depth record of every tag to identify periodicities (rhythmic patterns) (Graham et al. 2006). A FFT decomposes a time series record into component sine and cosine waves of different frequencies (periodicities), and the relative magnitude of the different periodic components is indicated by the size of the spectral peak in a power spectrum (Chatfield 2004). Results allow periodicities corresponding to tidal, diel, or longer term patterns of behavior to be identified (Graham et al. 2006; Shepard et al. 2006; Meyer et al. 2007).

Ambient light levels from the PAT archival records were used to divide each 24 h period into day or night. The inflection point in the dusk and dawn light curves from the WC-TSP output was used to delineate period of day. Where there were gaps in daily estimates of dusk and dawn, values were based on linear interpolation.

The thermal structure of the water column was recreated for the duration of each shark's track. Time series of temperature, time, and depth from the tags were used to create a linearly interpolated cross section of temperature across depth and time. This was implemented in Matlab using the TriScatteredInterp function (The Mathworks Inc.).

Depth and oceanographic data were summarized for each day of data from the archival record of every shark. We calculated daily estimates of mean and modal depth during the day, night, and entire day. Isothermal layer depth (ILD), a proxy for mixed-layer depth, was calculated for each day using the depth and temperature archival record and the method described in Kara et al. (2000) with a threshold value (ΔT) of 0.8 °C. Mean daily SST was estimated using the mean temperature of all temperatures recorded at depths $\leq$ 5 m.

There were three general patterns or behavioral modes apparent in the depth distribution of the sharks that generally occurred during different times of the year. Sharks were generally either shallow throughout the day (summer and early autumn), exhibited a diel shift in depth distribution in which they were shallow during the night and deeper during the day (autumn and early winter), or were generally deep throughout the day relative to the other two behavioral modes (winter and spring) (see Results: Vertical and thermal habitat). Every day was objectively assigned to one of these three behavioral

Table 2. Error in light-based longitude and SST-based latitude geolocation estimates.

	Error (km)	Error (°)	RMSE
Latitude	291.9 (330.5)	2.6 (2.9)	4.0
Longitude	51.2 (75.4)	0.9 (1.2)	1.5

Note: Mean and standard deviation of error (in kilometres and degrees) and root mean square error (RMSE) are presented. Estimates are based on difference between high-resolution Argos positions and geolocation estimates on corresponding days.

modes according to what each day's mean depth during the day and night was relative to the overall mean depth of that individual shark's depth record. Overall mean depth was very similar to overall median depth (mean difference, 8 m; minimum, 1 m; maximum, 18 m), and using median and mean depths produced very similar behavioral mode assignments.

A day was categorized as behavioral mode 1 (B1) when sharks were shallower than their overall mean depth during both day and night. A day was assigned to behavioral mode 2 (B2) when sharks were deeper than their overall mean depth during the day and shallower during the night. Behavioral mode 3 (B3) occurred when sharks were deeper than their overall mean during the day and night or were shallower than their overall mean during the day and deeper during the night. These two different patterns were grouped into B3 because the temporal distributions of these two patterns were not significantly different (Mann–Whitney U test, $w = 1.19$, $p = 0.23$) as they both primarily occurred during the winter and spring, whereas B1 primarily occurred during summer and B2 primarily occurred during autumn (see Results). In addition, during only 20% of B3 days were sharks shallower than their overall mean during the day and deeper during the night, so B3 primarily represents days when mean depth was deeper than the overall mean during the day and the night. Because of the short duration of the shorter archival records (sharks 097, 110, and 119), which reflected B1 behavior, behavioral mode assignments for these sharks were made using the grand mean of all salmon sharks' depth measurements (60.1 m) instead of the individual shark's mean depths, resulting in all days of data for these sharks being assigned to B1.

Once a behavioral mode had been assigned to every day, daily estimates of depth (mean and modal depth during day, night, entire day) and oceanographic (ILD, SST) parameters for all the sharks were aggregated by behavioral mode and visualized using box plots. Distributions of aggregated depth and oceanographic parameters during the different behavioral modes were then compared using a Mann–Whitney U test or Kruskal–Wallis test. Due to unequal sample size, we used the Dunn–Šidák correction for multiple comparisons (*Zar* 1999).

To describe diel patterns of water column and thermal environment use during the behavioral modes, proportion of time spent at depth and temperature by time of day was calculated. For every shark, time at depth (TAD) and time at temperature (TAT) were calculated at 15 min increments for every 24 h period and then aggregated by behavioral mode. The TAD and TAT data for all sharks were then combined to produce a single average TAD and TAT for B1, B2, and B3. Mean vertical displacement rate by depth was calculated for each behavioral mode in the same manner for shark 571, which was the only shark that had a PAT tag with a rapid enough sampling rate (5 s) to observe behavior with relatively high temporal resolution. Vertical displacement rate was calculated by dividing the change in depth between each record by the sampling rate. This rate was then assigned to the depth or depths between which the shark moved during the sample interval.

Results

Geographic distribution

Argos positions from SPOT tags indicate that the tagged sharks remained primarily in the neritic waters of Alaska during the duration of the PAT tag deployments (Fig. 2*a*), although some sharks migrated south from Alaskan waters after the PAT tags popped off. We use the term neritic to describe habitats between the outer shelf slope and the coast. Of the five sharks with longer PAT deployments, four migrated south and left the neritic waters of Alaska after the PAT tags popped off. This included sharks 931 (migrated in early February), 558 (mid-January), 111 (late February), and 571 (mid-April). Shark 087 remained in Alaskan waters throughout the year and migrated during the following February, approximately 18 months after tagging.

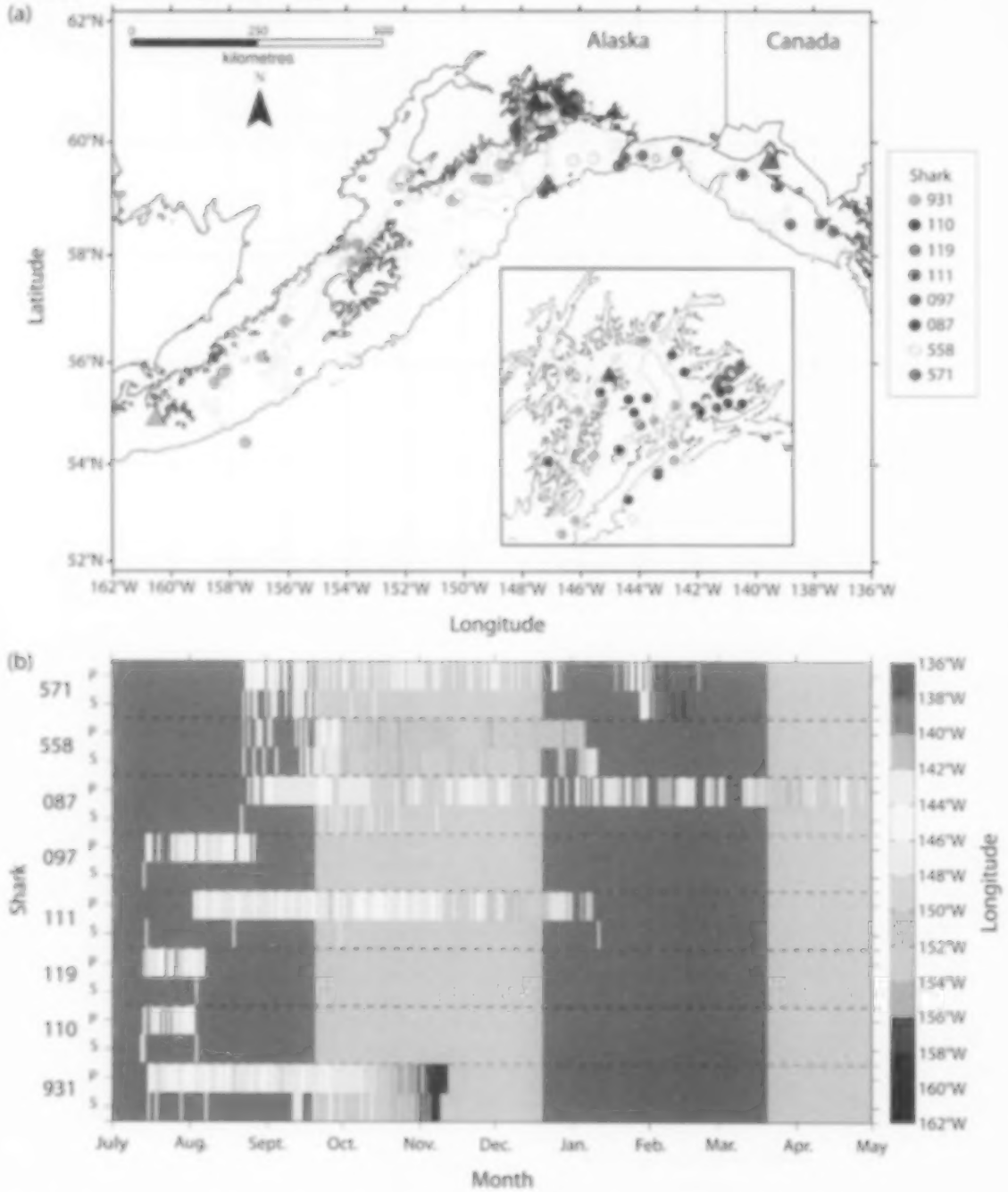
There were large gaps in the Argos records of all the sharks (Table 1; Fig. 2*b*). However, based on Argos positions and longitude estimates, sharks remained primarily in central Alaska (Figs. 2*a*, 2*b*), being most concentrated in the PWS region during the summer and more dispersed during the autumn, winter, and spring. In particular, sharks used primarily the region from Kodiak Island to PWS (roughly 154°W to 144°W). Longitude estimates and Argos positions suggest that the PWS region was used throughout the year but most intensively during the summer, though this may be an artifact of tagging location. Shark use of areas to the west of PWS (regions around the Kenai Peninsula and Kodiak Island) increased later in the year, especially during the autumn.

Vertical and thermal habitat

The depth distribution of the sharks changed in a consistent manner in relation to the water column thermal structure (Fig. 3*a*). During summer and early autumn, the water column was highly stratified, and sharks generally remained above the thermocline in warm, shallow waters throughout the day. As the water column stratification started to break down in the autumn, sharks exhibited a diel pattern of vertical migration in which they were generally deeper during the day and shallower during the night. In the winter and spring, sharks were deeper overall, and the water column became largely well mixed and cold. Inversion layers were present during the winter and spring as well (Fig. 3*a*), where it was colder at the surface than at depth. During the winter and spring, salmon sharks made their deepest dives (maximum 364 m in shark 087 during April) and encountered the coldest temperatures (2.5 °C at the surface in shark 087 during January), which primarily occurred in surface inversion layers. Sharks spent considerable lengths of time in cold waters; shark 087 spent at least 170 continuous days at temperatures below 8 °C and at least 80 continuous days at temperatures below 6 °C.

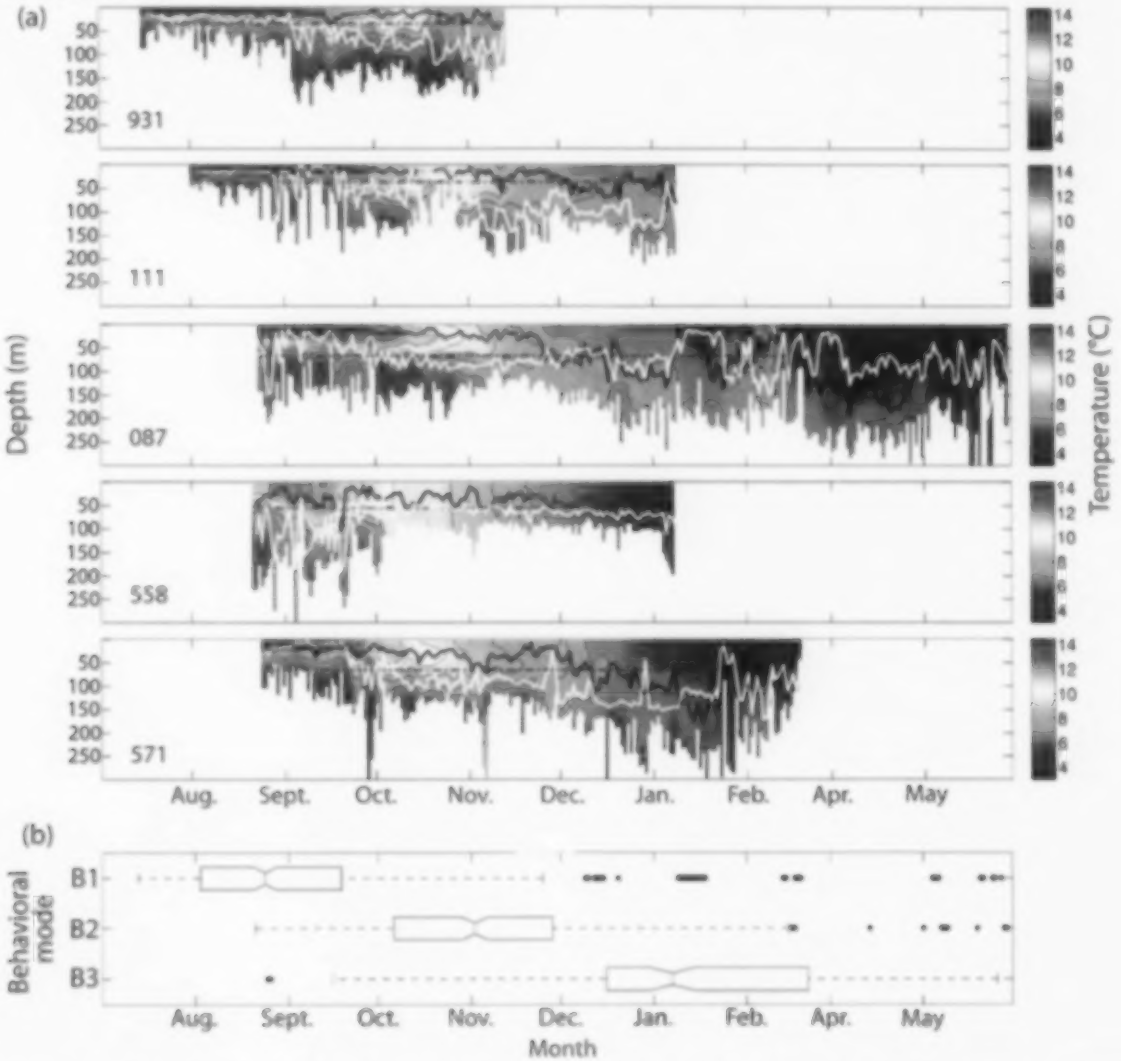
Spectral analysis (FFT) of archival depth records indicated

Fig. 2. (*a*) Northern Gulf of Alaska (with inset showing Prince William Sound, PWS) showing daily Argos positions from SPOT tags (circles) and pop-up location of tags (triangles), with positions color-coded by shark. Only Argos positions that overlap temporally with the PAT record are shown. The 200 and 300 m isobaths are also shown. (*b*) Time series of daily estimates of longitude based on geolocation (P) and SPOT positions (S) (color-coded by longitude) for each shark. Note the large gaps in the SPOT record and the high level of concordance between SPOT longitude and geolocation longitude.



that that the dominant periodicity in salmon shark depth was a diel (24 h) cycle (Fig. 4). The depth time series of all sharks had very large peaks at 24 h regardless of record length, indicating that the diel periodicity occurred throughout the duration of the PAT deployments (late summer through winter or early spring). Most sharks had smaller sec-

Fig. 3. (*a*) Time series of water column thermal structure experienced by sharks with tracks longer than 1.5 months. Water column thermal structure is color-coded by temperature, and bottom limit denotes daily maximum depth. Note that sharks rarely dove below 300 m, but these were truncated to make viewing the data clearer. Solid black lines denote mean depth during night; solid white lines denote mean depth during day; and broken black lines denote mean depth for entire track. Note that the maximum value in the color bar represents values ≥ that value. Shark numbers are listed in the lower left of each subplot. (*b*) Distribution of days assigned to behavioral modes 1 (B1), 2 (B2), and 3 (B3). Box denotes median and interquartile range (IQR, 25th to 75th percentiles); whiskers denote points within IQR × 1.5 of the ends of the box; points denote outliers.



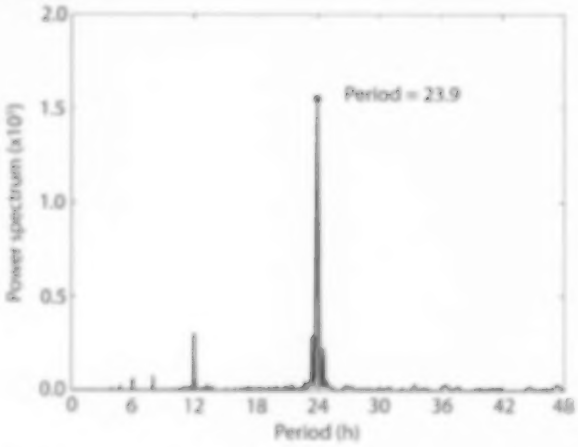
ondary peaks, most commonly at 12 h, perhaps indicative of crepuscular activity, but these were much weaker than the dominant peak.

Behavioral modes

We grouped the data into behavioral modes based on the three general patterns of vertical behavior (Fig. 5). The distribution of days assigned to the different behavioral modes showed a strong seasonal trend and reflected the observed seasonal changes in depth distribution (Fig. 3*b*). The temporal distributions of days assigned to the different behavioral modes were significantly different (Kruskal–Wallis test, H = 475.2, df = 2, $p < 0.001$). Days were generally categorized as B1 during the summer (367 B1 days; median date, 23 August; interquartile range (IQR), 2 August – 18 September). Days were typically categorized as B2 during the autumn (387 B2 days; median date, 1 November; IQR, 5 October – 27 November) and as B3 during the winter and spring (221 B3 days; median date, 6 January; IQR, 15 December – 21 February).

Although they were tagged over the course of 6 years, tagged salmon sharks exhibited consistent patterns in depth and temperature distribution during the different behavioral modes (Fig. 6). During B1 (summer), the depth range at

Fig. 4. Representative spectral analysis (FFT) of archival depth record of salmon sharks showing prominent diel (24 h) periodicity in depth. This FFT was generated from the archival record of shark 111.



which sharks spent the most time (focal depths) was in the warm, uppermost part of the water column (<10–15 m, 14–16 °C). Generally sharks spent little time below 50 m, though deeper dives generally occurred during the day. However, because of the high degree of stratification of the water column, sharks experienced a wide range of water temperatures (as cold as 4 °C) throughout the day, despite rarely going below 50 m. During B2 (autumn), sharks exhibited a strong diel pattern in which they used the warmer, shallower part of the water column (0–50 m, 7–11 °C) at night, descended at dawn to colder, deeper daytime depths (50–150 m, 5–7 °C), and then ascended to shallower nighttime depths at dusk. During B3 (winter and spring), sharks used more of the water column than during other times of the year, especially during the night. Although sharks were still more concentrated in the upper 100 m of the water column at night, they had the widest depth distribution and regularly dove to greater depths during the night, and their use of the shallowest part of the water column during the night also diminished greatly. During the day, their depth range was similar to what they used during B2. In the largely unstratified water column that exists during the winter and spring, the sharks were primarily restricted to temperatures between 5 and 7 °C throughout the day, despite occurring over a greater range of depths.

The depth distribution of sharks changed between behavioral modes, and there were significant diel differences in depth (Figs. 7*a*, 7*b*), as was indicated by the FFT analysis. Mean daily depth increased significantly from B1 to B2 to B3 (median of daily mean depths during B1, 15.5 m; B2, 52.0 m; B3, 91.2 m; Kruskal–Wallis test, $H = 736.8$, df = 2, $p < 0.001$). During B1, the sharks were generally shallow throughout the day, but daytime depths were significantly deeper than during the night (median of mean daytime depths, 15.7 m; nighttime depths, 11.5 m; Mann–Whitney U test, $w = 43.3$, $p < 0.001$; Fig. 7*a*). The diel change in depth distribution was clear during B2 (Fig. 7*a*), with nighttime depths being significantly shallower than daytime depths (median daytime depths, 81.5 m; nighttime depths, 24.6 m; Mann–Whitney U test, $w = 550.4$, $p < 0.001$; Fig. 7*a*). There was no significant diel difference in the depths of sharks during B3 (median daytime depths, 90.7 m; nighttime depths, 91.7 m; Mann–Whitney U test, $w = 1.1$, $p = 0.284$; Fig. 7*a*).

Daily estimates of modal depth showed a similar pattern during the different behavioral modes as well (Fig. 7*b*). Daily modal depths increased significantly from B1 to B2 to B3 (median of daily modal depths during B1, 5 m; B2, 10.0 m; B3, 70 m; Kruskal–Wallis test, df = 2, $H = 219.9$, $p < 0.001$). The modal daytime and nighttime depths were significantly different during B1 (Mann–Whitney U test, $w = 34.8$, $p < 0.001$) and B2 (median of daytime depths, 5 m; nighttime depths, 90 m; Mann–Whitney U test, $w = 357.9$, $p < 0.001$; Fig. 7*b*,), although as opposed to mean depth, B3 was also significantly different (median daytime depths, 85 m; nighttime depths, 50 m; Mann–Whitney U test, $w = 18.58$, $p < 0.001$; Fig. 7*b*). The diel periodicity in depth identified using mean and modal depths is supported by the results of the spectral analysis, which indicated that there was a clear diel periodicity throughout the duration of the tracks. Modal depth appears to better reflect the diel pattern exhibited during B3 (Fig. 6), with sharks being deeper overall but still slightly shallower during the night than during the day overall. This highlights the importance of selecting the appropriate depth metric when examining depth distribution and how spectral analysis can inform your data analysis and interpretation.

The oceanographic conditions during the different behavioral modes also showed a clear change reflecting the seasonal nature of the behavioral modes (Figs. 7*c*, 7*d*). Sea surface temperature differed significantly between all of the behavioral modes (Kruskal–Wallis test, $H = 544.6$, df = 2, $p < 0.001$), with SST being the warmest during B1 (median of mean daily SST, 14.9 °C) and the coldest during B3 (median, 5.6 °C) (Fig. 7*c*). Isothermal layer depth decreased significantly from B1 (median, 13.2 m) to B2 (median, 55.8 m) (Kruskal–Wallis test, $H = 339.5$, df = 2, $p < 0.001$), but not from B2 to B3 (median, 94.7 m) (Fig. 7*d*). As the ILD deepened over the course of the year, the number of days with water columns categorized as isothermal (no bottom of the ILD was identified up to the maximum depth that the shark occupied) increased, as did the number of days during which an inversion layer was present. During B1, the water column was isothermal on 14.4% of the days and had an inversion layer on 4.4% of the days. During B2, 35.8% of the days were isothermal and 10.7% had an inversion layer. During B3, 92.2% of the days had isothermal water columns and 72.6% had an inversion layer. Many of the days during B1 and B2 were categorized as isothermal due to sharks remaining very shallow (above the ILD) rather than indicating a deep ILD as was the case during B3. The lack of a significant difference in ILD between B2 and B3 was due to so many days during B3 being categorized as isothermal.

As the isothermal layer deepened over the course of the year, residency time within the isothermal layer also generally increased. During B1, median percentage of time spent within the ILD was 70.5% (IQR 42.3%–96.8%) during the day and 90.7% (IQR 63.3%–99.7%) during the night. During B2, median percentage of time spent in the ILD decreased to 48.2% (IQR 22.3%–99.8%) during the day but increased to 99.8% (IQR 96.8%–100%) during the night. Sharks spent nearly 100% of their time in the ILD during both the day

Fig. 5. Representative depth time series showing behavior during (*a*) B1, (*b*) B2, and (*c*) B3. Data are from shark 571. The background water column is color-coded by temperature, and nighttime is shaded gray.

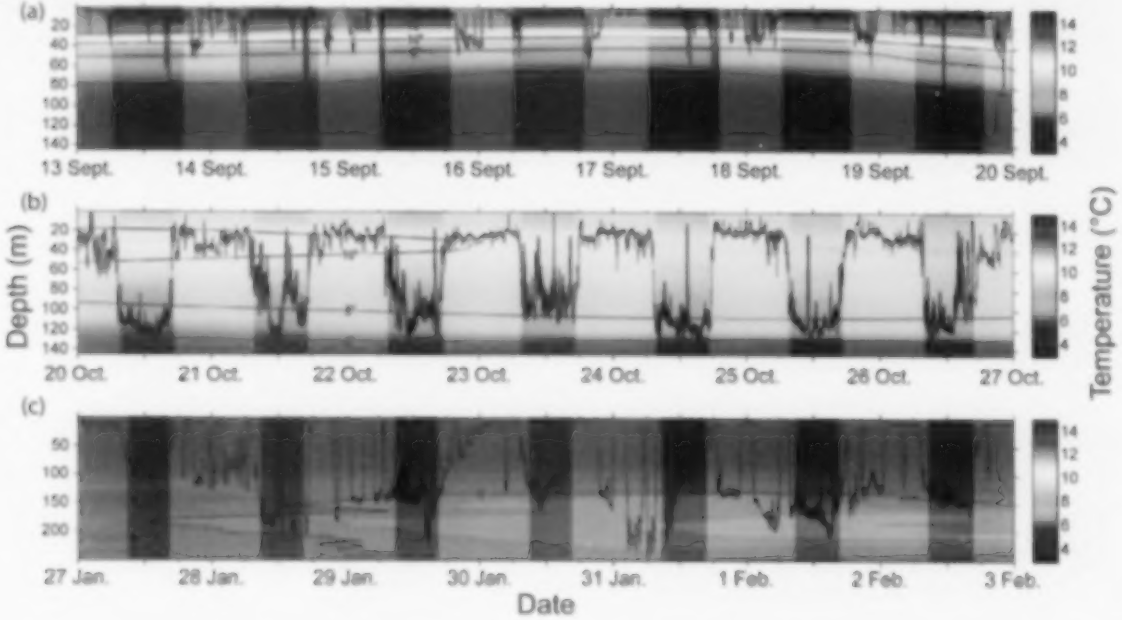
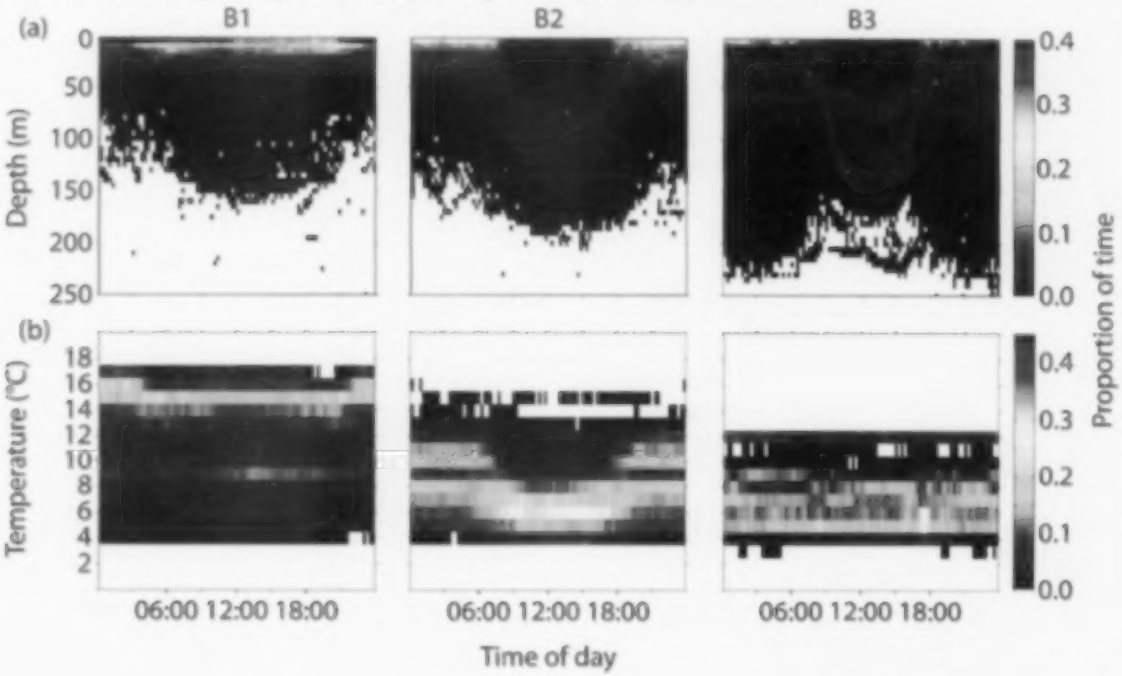


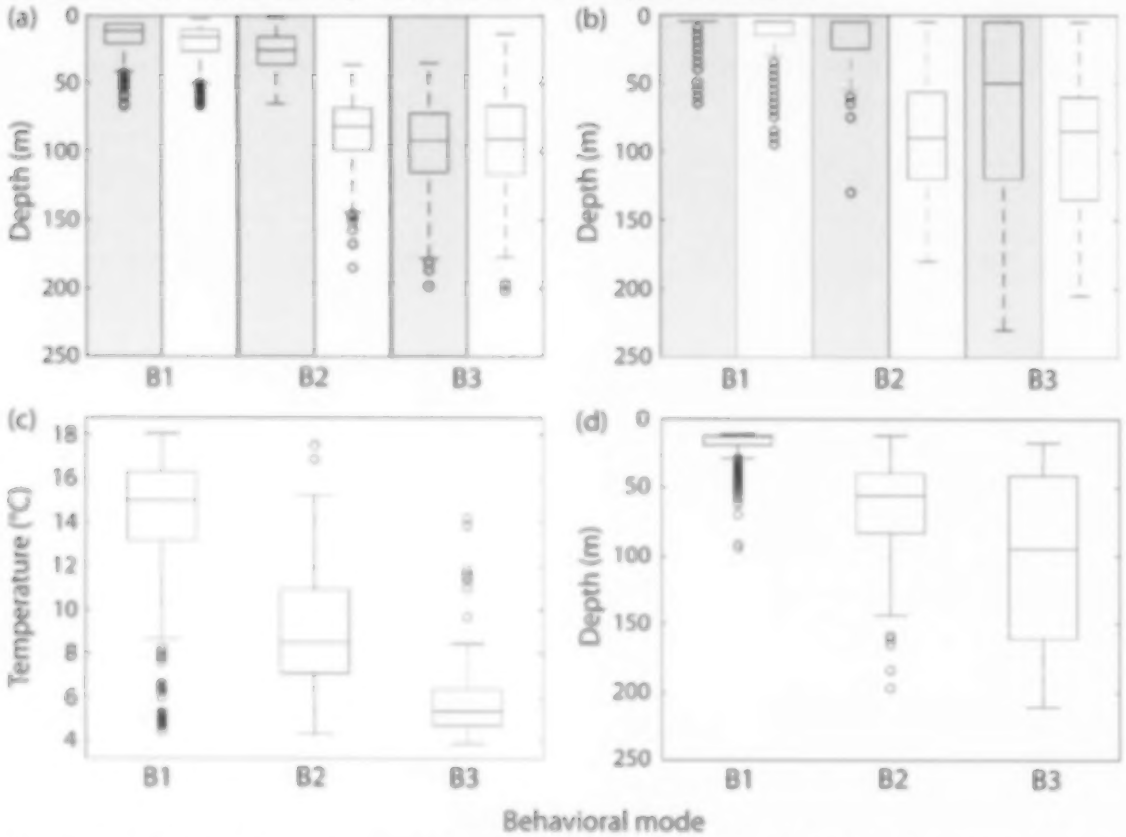
Fig. 6. (*a*) Mean time at depth (TAD) and (*b*) mean time at temperature (TAT) by time of day of all sharks during each behavioral mode. Plots are color-coded by proportion of time. Note that the maximum value in the color bar represents values ≥ that value. The dotted red line in B2 in (*a*) shows mean depth of capture of salmon sharks by commercial fisheries (see Discussion).



and night during B3 because of the lack of thermal stratification in the water column.

Shark 571 generally exhibited more rapid vertical movements during the day and at the bottom of its depth distribution during each behavioral mode (Fig. 8). During B1, the shark's vertical movements were faster during the day than

Fig. 7. Distribution of daily (*a*) mean and (*b*) modal depths by period of day during the different behavioral modes, with nighttime depths being shaded. (*c*) Distribution of daily SST during the different behavioral modes. (*d*) Distribution of daily isothermal layer depth (ILD) during the different behavioral modes; note that there were far fewer days when ILDs were identified during B3 due to the presence of temperature inversions and the frequency of the water column being isothermal. Box denotes median and interquartile range (IQR, 25th to 75th percentiles); whiskers denote points within IQR × 1.5 of the ends of the box; points denote outliers.



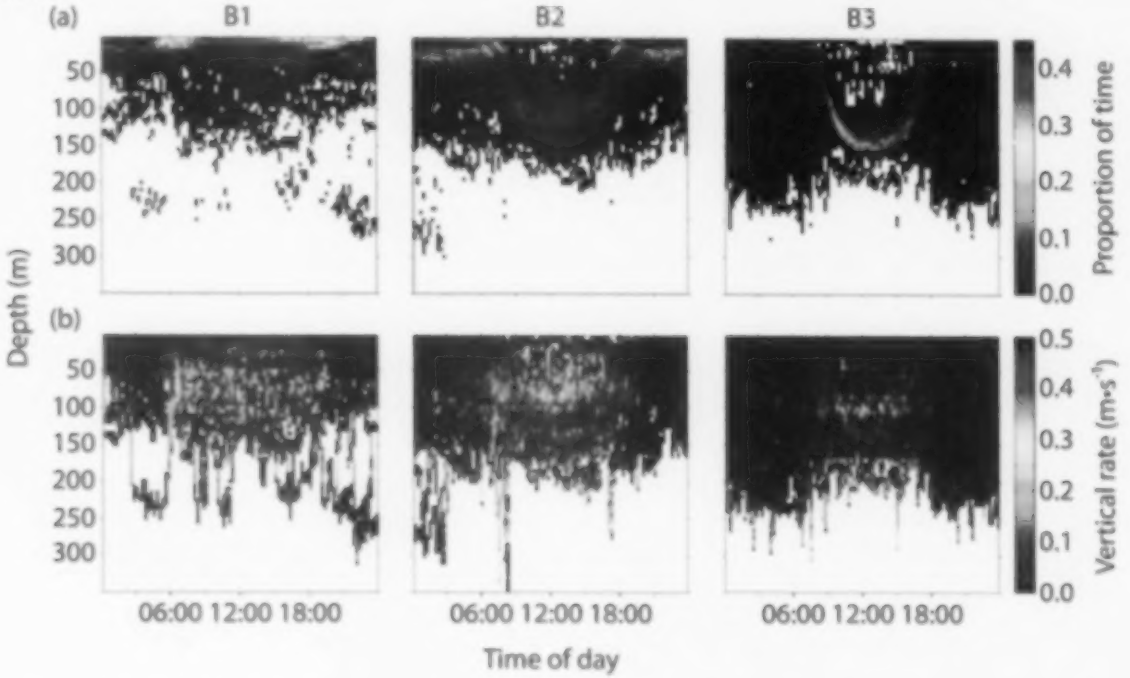
at night (day: mean, 0.13 ± 0.13 m·s^{-1}; maximum, 1.70 m·s^{-1}; night: mean, 0.09 ± 0.05 m·s^{-1}; maximum, 0.50 m·s^{-1}), and in general, most of the rapid dives occurred below about 50 m and vertical displacement rate was generally low near the surface. There was a similar pattern during B2 in which the shark again exhibited more rapid vertical movements during the day and at the bottom of its depth distribution (day: mean, 0.10 ± 0.13 m·s^{-1}; maximum, 1.53 m·s^{-1}; night: mean, 0.08 ± 0.05 m·s^{-1}; maximum, 0.65 m·s^{-1}). Most of the rapid vertical daytime movements occurred above and below focal depths. During B3, the vertical displacement rate of the shark was more consistent across depths and time (day: mean, 0.10 ± 0.07 m·s^{-1}; maximum, 1.35 m·s^{-1}; night: mean, 0.07 ± 0.04 m·s^{-1}; maximum, 0.7 m·s^{-1}). However, the shark still exhibited the highest vertical displacement rates during the day and at the bottom of its depth distribution, and the highest vertical displacement rates were at depths just above daytime focal depths.

Discussion

The results of this study indicate that some female salmon sharks reside in the coastal Alaskan waters of PWS and adjacent neritic habitats throughout the winter and that their movements and behaviors shift with seasonally changing oceanographic conditions. Furthermore, the vertical distribution of the salmon sharks tagged in this study changed in a predictable and consistent manner, information that may be useful in reducing fisheries interactions with this important predator in neritic habitats. Changes in the depth distribution of salmon sharks likely represent shifts in their foraging ecology and, thus, possible interactions with commercially important species such as Pacific salmon, walleye pollock, and Pacific herring (*Clupea pallasi*).

Changes in the depth distribution of salmon sharks were associated with seasonal oceanographic conditions. Specifically, we identified three distinct behavioral modes that represent shark behavior during the summer – early autumn (B1), autumn (B2), and winter – spring (B3). Oceanographic conditions during the different behavioral modes were distinct, suggesting that salmon sharks distribute themselves vertically according to three different oceanographic seasons. Oceanographic conditions recorded by PAT tags closely matched seasonal oceanographic changes reported in the literature for the northern GOA (Wang et al. 2001; Royer 2005; Sarkar et al. 2005). The shifts between behavioral modes are most

Fig. 8. (*a*) Mean time at depth (TAD) and (*b*) vertical displacement rate by depth and time of day of shark 571 during each behavioral mode. Note the different color bars for (*a*) proportion of time and (*b*) vertical displacement rate. Note that the maximum value in the color bar represents values ≥ that value; values greater than 0.5 m·s^{-1} for (*b*) were truncated to more clearly show the pattern, and higher values were observed.



likely a response to changes in prey distribution and availability during distinct oceanographic periods.

Due to the specialized anatomical and physiological traits possessed by *Lamna* that allow them to maintain elevated body temperatures and essentially function as homeotherms (Goldman et al. 2004), the influence of water temperature on their movements and behaviors is likely moderated (Weng et al. 2008). Salinity reaches its lowest levels in the top 20–30 m of the water column (25 ppt at the surface to 29.5 ppt at 30 m) in the northern GOA during late summer and autumn (Royer 2005) when salmon sharks use these depths extensively, indicating they are not greatly impacted by reduced salinity. Therefore, it is likely that the influence of oceanographic conditions on the distribution of salmon sharks in the northern GOA is primarily mediated through its impact on the abundance and distribution of important prey species such as Pacific salmon, walleye pollock, and squid (Teuthoidea), which are ectothermic and may be more directly affected by oceanographic conditions such as temperature. This suggests that seasonal shifts in salmon shark depth distribution primarily represent changes in foraging behavior.

The region used by tagged sharks, which encompassed primarily areas of the continental shelf from east of PWS (including PWS itself) to west of Kodiak Island (approximately 144°W to 156°W), corresponds with areas used by salmon sharks identified by Weng et al. (2005) and Hulbert et al. (2005). This region has been reported to be highly productive (Feder and Jewett 1986; Sambrotto and Lorenzen 1986; Mueter and Norcross 2002), supporting a diversity and abundance of potential prey items (Mueter and Norcross 2002; Mundy and Hollowed 2005; Jorgensen 2007). Overall, the western GOA is characterized as having relatively higher productivity supporting a high biomass and lower diversity of fishes, and the eastern GOA is characterized as being relatively less productive, with a lower biomass and higher diversity of fishes (Mueter and Norcross 2002; Mundy and Hollowed 2005).

Several species known to be important salmon shark prey (Nagasawa 1998; Hulbert et al. 2005; Goldman and Musick 2008) such as Pacific salmon (Groot and Margolis 1991; Myers et al. 2000), walleye pollock, and Pacific herring (Hay and McCarter 1997; Mundy and Hollowed 2005) are abundant in the neritic habitats of central Alaska used by tagged salmon sharks. In particular, walleye pollock, which are one of the most abundant species of fish in the GOA and very important ecologically and economically (walleye pollock support the largest single-species commercial fishery in the world) (Springer 1992; Yang and Nelson 2000; Mueter and Norcross 2002), are generally found at their highest densities in the GOA in inner shelf habitats (100–200 m deep, within 20–40 nm of land), between 147°W and 154°W (though they are abundant to 170°W) (Brodeur and Wilson 1996*b*; Kendall et al. 1996; Shima et al. 2002). The distribution of cephalopods in the GOA has not been described, but they are the largest component of nontarget catch in the pollock fishery in the western and central GOA (Ormseth and Gaichas 2009) and are believed to be abundant in pelagic waters surrounding the outer continental shelf and slope (Sinclair et al. 1999).

The increased productivity and the diverse and abundant food web that coastal GOA and adjacent waters support likely underlie the extensive use of this region by salmon sharks. This region also corresponds to the area of the GOA in which the greatest numbers of salmon sharks are caught as nontarget catch in commercial fisheries. The region spanning from Kodiak to PWS saw an estimated 380.8 tonnes of salmon sharks (55% of total estimated catch in GOA) caught as reported, nontarget catch in commercial fisheries between 1997 and 2008, with the area between Kodiak and the Shumagin Islands having the next highest level of bycatch (164.4 tonnes or 24% of the catch) (Tribuzio et al. 2008).

Seasonal shifts in depth distribution: behavioral mode 1

The vertical distribution of the tagged sharks provides further information about foraging behavior and how this shifts over time in a dynamic and changing ocean. Caution is always warranted when drawing conclusions from a small sample size. However, the consistency of the patterns that we describe across individuals and the 6 years of tagging suggest that our results are representative.

The geographic and vertical distribution of tagged salmon sharks during the summer and early autumn (B1) likely reflects the distribution of Pacific salmon and represents foraging on salmonids. Salmon sharks have been reported to use PWS extensively during the summer and early autumn and to form large aggregations in some of its bays and fjords to feed on the aggregations of Pacific salmon that gather near the mouths of their natal rivers in PWS prior to making their upstream spawning migrations (Paust and Smith 1989; Hulbert et al. 2005; Weng et al. 2005). The major component of salmon shark diet in PWS during the summer is pink (*Oncorhynchus gorbuscha*), chum (*Oncorhynchus keta*), and coho (*Oncorhynchus kisutch*) salmon (Hulbert et al. 2005), although sharks still consumed a variety of other prey (squid and teleosts such as sablefish (*Anoplopoma fimbria*) and Pacific herring) despite high local densities of salmon (Hulbert et al. 2005).

Our results indicate that in summer months, salmon sharks likely forage primarily on salmon in shallow surface waters throughout the diel cycle. The shallow distribution of salmon sharks and slight diel shift in depth during B1 closely matches what has been reported for salmon. Many studies have documented diel vertical migration in Pacific salmon (Manzer 1964; Walker et al. 2006, 2007). Walker et al. (2006) reported that pink, coho, and chum salmon primarily remained in the upper 20 m of the water column (with occasional deeper excursions) and that their average depth was slightly deeper during the day (12–20 m) than during the night (4–8 m).

However, salmon sharks also forage on nonsalmonid prey (squid, demersal or benthic teleosts) during this period (Hulbert et al. 2005; K.J. Goldman, unpublished data), and it is likely that this occurs during the day. Sharks were slightly deeper during the day and made the deepest dives during the day, and the vertical displacement rate was highest during the day at depths below 50 m, suggesting that sharks may be more actively foraging in deeper waters during their brief daytime vertical excursions from shallow waters. Chum salmon have also been reported to move faster during dives below the thermocline, possibly while in pursuit of more active fish prey distributed below the thermocline (Tanaka et al. 2005). These deeper daytime dives may represent foraging on benthic or demersal prey as these dives are deeper than the depths typically used by salmon (Manzer 1964; Walker et al. 2006; Walker et al. 2007). That the vertical displacement rate was very low at the depths at which the shark was most concentrated (<50 m) suggests that any increased activity was in the horizontal dimension, and indeed salmon sharks are frequently observed rapidly pursuing salmon in shallow surface waters (authors, personal observations).

The behavioral shift from B1 to B2 during autumn coincides temporally with the end of the salmon runs. This suggests that the foraging ecology of the tagged salmon sharks shifted from one focused primarily on aggregating salmon to one that capitalizes on other abundant prey items or a wider variety of prey. It is important to note that these results for B1 are primarily applicable to sharks that aggregate in PWS or other bays during the summer months to feed on salmon.

Seasonal shifts in depth distribution: behavioral mode 2

After the salmon runs end, the salmon shark aggregations disperse and tagged sharks spent more time in the neritic habitats between Kodiak Island and PWS as water temperatures cooled and the water column became less stratified. Their behavior shifted from being surface-oriented to having a strongly diel pattern during B2 (autumn) that is typical of diel vertical migration (DVM). This shift in salmon shark behavior suggests that sharks started foraging on prey items that exhibit diel vertical migration, which is common to a large number of marine organisms (Roper and Young 1975; Neilson and Perry 1990; Hays 1996). Diel vertical migration has been observed in a number of elasmobranchs, including the salmon shark's congener, the porbeagle (Pade et al. 2009), and has typically been associated with the tracking of prey (Weng and Block 2004; Graham et al. 2006; Shepard et al. 2006). Hulbert et al. (2005) and Weng et al. (2005) showed a general bimodal depth distribution in salmon sharks during the autumn and winter. Although these patterns were not specifically addressed there, they likely reflect the diel pattern that we have described.

Although the diet of salmon sharks in coastal Alaska outside of the period of the summer salmon runs has not been described, there are a variety of organisms known to be consumed by salmon sharks that also exhibit DVM, including Pacific herring (Hay and McCarter 1997; Mackinson 1999), capelin (*Mallotus villosus*) (Mowbray 2002; Wilson et al. 2003), pollock (Brodeur and Wilson 1996*a*; Adams et al. 2007; Adams et al. 2009), salmon (Walker et al. 2007), and squid (Teuthoidea) (Roper and Young 1975; Watanabe et al. 2006). Salmon exhibit DVM; however, the extent of their vertical movements is much more limited and primarily restricted to the upper 20–40 m (Walker et al. 2006), suggesting that this behavior is not primarily related to salmon. In addition, salmon (especially pink, chum, and sockeye) generally move offshore during the autumn to more oligotrophic waters where they remain before moving back inshore during the spring (Nagasawa 2000; Quinn 2005), so their geographic distribution may overlap with salmon sharks in neritic habitats less during the autumn and winter.

Walleye pollock exhibit DVM and are likely an important forage species for salmon sharks in neritic habitats through-

out the year, especially during the autumn, winter, and spring (B2 and B3). Pollock are known to vertically migrate from deeper diurnal depths to shallow nocturnal depths to forage (Brodeur and Wilson 1996*a*; Wilson et al. 2003; Adams et al. 2009). In the GOA, juvenile pollock rise in the water column at night to form diffuse layers concentrated at shallow depths (10–40 m) to forage (Brodeur and Wilson 1996*a*; Adams et al. 2007) and descend to deeper depths (around 90–150 m) in the daytime where they form tight ball-like aggregations (Brodeur and Wilson 1996*a*; Wilson et al. 2003), likely as an antipredation tactic (Brodeur and Wilson 1996*b*). Although adults vertically migrate at times (Miyashita et al. 2004; Adams et al. 2007, 2009), it is believed that they are primarily found in loose demersal aggregations throughout the day (Wilson et al. 2003). Both adults and juveniles generally remain within 30 m of the bottom during the day, though juveniles typically are shallower than the adults (Brodeur and Wilson 1996*a*; Wilson et al. 2003). These patterns of depth distribution are remarkably similar to those observed in the tagged salmon sharks.

The likely importance of pollock to salmon sharks throughout the year is supported by the fact that most of the reported nontarget catch of salmon sharks is in the commercial pollock fishery (Tribuzio et al. 2008). Approximately 73% of the total reported nontarget catch of salmon sharks in the GOA between 1997 and 2008 was in the pollock fishery (yearly average, 78%; minimum, 65%; maximum, 98%). An estimated 357.2 tonnes of salmon sharks were caught between 1997 and 2008 in pelagic pollock trawls, accounting for 57% of the total reported salmon shark catch in commercial fisheries during that period (yearly average, 59%; minimum, 12%; maximum, 98%). Bottom pollock trawls caught an estimated 102.4 tonnes of salmon sharks between 1997 and 2008, or 16% of the total reported catch (yearly average, 19%; minimum, 0%; maximum, 66%) (Tribuzio et al. 2008).

Further evidence that salmon sharks forage on pollock is that their daytime focal depths during B2 and B3 were similar to the depth of trawls that caught salmon sharks as nontarget catch in commercial fisheries (primarily the pollock fishery). Based on National Marine Fisheries Service (NMFS) Alaska groundfish observer data from the GOA, the average fishing depth of commercial pelagic trawls (aggregated by NMFS area and quarter and weighted by number of hauls) between 1993 and 2009 in which salmon sharks were caught was 135 m (minimum, 52 m; maximum, 249 m), whereas the average depth of bottom trawls in which salmon sharks were caught was 137 m (minimum, 65 m; maximum, 241 m) (Ren Narita, Fisheries Monitoring and Analysis Division, NOAA-NMFS, Alaska Fisheries Science Center, 7600 Sand Point Way N.E., Building 4, Seattle, WA 98115, USA, personal communication, 2009). In addition, a female shark (197 cm FL) tagged during this project was recaptured together with a male salmon shark (186 cm FL) in Shelikof Strait in March 2008 by the NOAA ship *Oscar Dyson* in a research pollock trawl (Abigail McCarthy, Resource Assessment and Conservation Engineering Division, NOAA-NMFS, Alaska Fisheries Science Center, 7600 Sand Point Way N.E., Building 4, Seattle, WA 98115, USA, personal communication, 2008). The depth of this trawl (150 m in 4.3 °C water) was similar to the depths at which salmon sharks concentrated during the day during B2 and B3.

Other potential prey species that exhibit DVM and are known to be fed upon by salmon sharks include Pacific herring. Salmon sharks forage extensively on Pacific herring at times (Blagoderov 1994; Hulbert et al. 2005) and are in fact known as "herring sharks" in Russia (Blagoderov 1993). Indeed, the tag on shark 571 popped off inside Yakutat Bay in mid-February in the middle of one of the largest herring aggregations in recent memory (Geoff Widdows, Sea Raven Charters, P.O. Box 342, Yakutat, AK 99689, USA, personal communication, 2008). Pacific herring also exhibit DVM (Hay and McCarter 1997; Mackinson 1999; Nilsson et al. 2003). Capelin follow a similar vertical migration pattern as juvenile pollock and often are mixed together with dispersed layers of pollock (Brown 2002; Wilson et al. 2003). Cephalopods are well known vertical migrators (Roper and Young 1975; Watanabe et al. 2006), and salmon sharks are known to forage intensively on them in oceanic (Kubodera et al. 2007) and coastal (Hulbert et al. 2005) habitats. Stomach contents of salmon sharks caught in spring and early summer as they come into PWS from the GOA were dominated by squid (K.J. Goldman, unpublished data).

Shark 571 did not exhibit rapid vertical movements at its daytime focal depths but exhibited an increased vertical displacement rate above and below its focal depths. It is possible that this reflects foraging behavior such as pursuit of demersal prey up into the water column or down to the bottom. Alternatively, the increased vertical displacement rate above daytime focal depths may reflect active searching of the water column, and rapid movements during deep dives could represent dives to consume benthic prey.

Whether or not salmon sharks are following particular vertically migrating prey, following the vertically migrating assemblage, or accessing different prey at different times of the day is unclear and impossible to ascertain from tag data alone. It is likely that they forage on a diversity of prey organisms, although some prey such as pollock, herring, and squid are likely of increased importance. The frequency with which salmon sharks and squid are caught as nontarget catch in the pollock fishery indicates that these three species often co-occur, further evidence that squid and pollock are likely important prey species for salmon sharks during B2 and B3.

Seasonal shifts in depth distribution: behavioral mode 3

During B3 (winter and spring), tagged salmon sharks still primarily occurred in neritic habitats of central Alaska in a largely unstratified, very cold ocean that often had temperature inversions. During B3, sharks spent the least amount of time at very shallow depths relative to B1 and B2 (hence the lack of Argos detections during winter and spring), and sharks used the widest range of depths at night. The shift in salmon shark depth distribution during B3 may be attributable to physiological limitations in the shark and (or) a shift in the distribution of prey.

The coldest temperatures (as low as 2.5 °C) experienced by salmon sharks were primarily in the surface inversion layer during the winter and spring, when salmon sharks used shallow habitats the least and overall occupied deeper depths, suggesting that thermal physiological limitations may become a factor during this period. Shallow waters (<50 m) reach their lowest temperatures (<5 °C) from January–April, when temperatures could be as much as ~1–2 °C colder than those

that occur at deeper depths (Royer 2005). Thus the increased use of deeper depths during the period of temperature inversions suggests that warmer waters at depth may provide a limited thermal refuge for salmon sharks (or their prey) during this period. Paust and Smith (1989) suggested that salmon sharks may be found along with other epipelagic organisms in thermal refuges beneath cold surface waters during the winter.

In addition to possible physiological limitations, the spatial and vertical distribution of prey likely changes during B3 in response to changing oceanographic conditions as ectothermic species would be more sensitive to temperature changes than salmon sharks. Although pollock exhibit some degree of DVM throughout the year (Adams et al. 2007, 2009), the degree of DVM appears to be reduced during the winter and spring as pollock remain closer to the bottom throughout the day and are generally found in deeper waters (Brodeur and Wilson 1996*a*). Overall, the nearshore demersal fish assemblage moves to deeper waters during the winter (Mundy and Hollowed 2005).

Despite the increase in nighttime depth distribution, shark 571 moved at a relatively steady vertical rate through the water column, although depth distribution and vertical displacement rate during the day generally followed a similar pattern as in B2. This may indicate foraging on a prey field that is more dispersed throughout the water column during the night. This might occur following a reduction in vertical migration in prey such as occurs in pollock. During the day, salmon sharks would still have access to demersal aggregations of prey.

Why some female salmon sharks overwinter in Alaska and do not migrate to southern regions is unclear. It has been suggested that female salmon sharks migrate to lower latitudes to give birth (Nagasawa 1998; Weng et al. 2008). If this is the case, it is possible that female sharks that remain at high latitudes throughout the year (e.g., shark 087) do not migrate because they are nonreproductive (immature, in a reproductive resting stage, or have been unsuccessful in mating). One would expect sharks that are not bound by reproductive (i.e., the need to migrate to pup and (or) mate) or physiological (i.e., need to leave high latitudes because of cold temperatures) constraints to utilize regions that provide them with high-quality resources.

The fact that some salmon sharks exploit the highly productive cold waters of the North Pacific throughout the year indicates that the amount of energy available to them in productive boreal waters compensates for the increased metabolic cost associated with using these cold habitats during the winter and spring. By being able to take advantage of locally available and abundant prey such as salmon, pollock, and squid, the sharks are able to successfully exploit a seasonally shifting prey field across a range of oceanographic conditions. This opportunistic foraging strategy, coupled with their endothermic capacity and cardiac physiology, allows salmon sharks to successfully exploit boreal waters throughout the year. Their unique anatomy and physiology is likely a great advantage when foraging on ectothermic prey and competing with other ectothermic predators in cold temperatures, and indeed, the endothermic capability of salmon sharks is believed to underlie their evolutionary expansion into boreal waters (Block and Finnerty 1994; Goldman et al. 2004; Weng et al. 2005). Their ability to use boreal waters gives them access to an abundance of prey and may also free them of competitive constraints that they may experience at lower latitudes from other large predatory fishes and elasmobranchs that are unable to as efficiently use these cold habitats because of their lack of endothermic capacity.

The spatial and vertical distribution of salmon sharks in the GOA overlap with a number of commercially important species, and as a result, the species is commonly caught as nontarget catch in a number of commercial fisheries (Camhi 1999; Goldman and Musick 2008). Although the impact of this removal on their population is unclear, it may be an important source of mortality. Our results suggest that given the consistent seasonal patterns of geographic and vertical distribution of salmon sharks in the GOA, measures taken to reduce gear interaction may be effective at limiting the number of sharks caught as nontarget catch and reduce the impact of commercial fishing on salmon shark populations.

Given the susceptibility of sharks to overexploitation (Musick et al. 2000) and the important ecological role that salmon sharks likely play in North Pacific ecosystems as the largest apex predator in these systems (Goldman and Musick 2008), they need to be managed responsibly. Furthermore, their possible role as important sources of natural mortality to a variety of commercially important species should be assessed and accounted for in stock assessments and management plans.

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Interactions between natural-occurring landscape conditions and land use influencing the abundance of riverine smallmouth bass, *Micropterus dolomieu*

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Abstract: This study examined how interactions between natural landscape features and land use influenced the abundance of smallmouth bass, *Micropterus dolomieu*, in Missouri, USA, streams. Stream segments were placed into one of four groups based on natural-occurring watershed characteristics (soil texture and soil permeability) predicted to relate to smallmouth bass abundance. Within each group, stream segments were assigned forest ($n = 3$), pasture ($n = 3$), or urban ($n = 3$) designations based on the percentages of land use within each watershed. Analyses of variance indicated smallmouth bass densities differed between land use and natural conditions. Decision tree models indicated abundance was highest in forested stream segments and lowest in urban stream segments, regardless of group designation. Land use explained the most variation in decision tree models, but in-channel features of temperature, flow, and sediment also contributed significantly. These results are unique and indicate the importance of natural-occurring watershed conditions in defining the potential of populations and how finer-scale filters interact with land use to further alter population potential. Smallmouth bass has differing vulnerabilities to land-use attributes, and the better the natural watershed conditions are for population success, the more resilient these populations will be when land conversion occurs.

Résumé : Notre étude examine comment les interactions entre les caractéristiques du paysage naturel et l'utilisation des terres influencent l'abondance des achigans à petite bouche, *Micropterus dolomieu*, dans des cours d'eau du Missouri, É.-U. Les segments de cours d'eau ont été répartis en quatre groupes d'après les caractéristiques naturelles de leur bassin versant (texture et perméabilité des sols) présumées être en relation avec l'abondance des achigans à petite bouche. Dans chaque groupe, les segments de cours d'eau ont reçu des étiquettes de forêt ($n = 3$), de pâturage ($n = 3$) ou de zone urbaine ($n = 3$) d'après les pourcentages d'utilisation des terres dans chaque bassin versant. Des analyses de variance montrent que les densités d'achigans à petite bouche varient en fonction de l'utilisation des terres et des conditions naturelles. Des modèles d'arbres de décision indiquent que l'abondance est maximale dans les segments de cours d'eau en forêt et minimale dans les segments de cours d'eau urbains, quelle que soit la désignation du groupe. L'utilisation des terres explique le plus de variation dans les modèles d'arbres de décision, mais les caractéristiques internes du chenal, température, débit et sédiments, contribuent aussi de manière significative. Ces résultats sont inédits et ils indiquent l'importance des conditions naturelles du bassin versant pour définir le potentiel démographique; ils montrent comme les filtres à échelles plus fines interagissent avec l'utilisation des terres pour modifier encore plus le potentiel démographique. Les achigans à petite bouche possèdent des vulnérabilités différentes aux diverses caractéristiques de l'utilisation des terres; plus les conditions naturelles du bassin versant sont favorables au succès de la population, plus la population sera résiliente lorsqu'il se produira une modification dans l'utilisation des terres.

[Traduit par la Rédaction]

Introduction

Recognition of the vast extent and ecological importance of land-use changes worldwide (Turner and Meyer 1994) has resulted in increasing numbers of studies examining effects of land-use changes on aquatic systems (e.g., Marshall et al. 2008; Utz et al. 2010; Yates and Bailey 2010). Landscape conversions from forest or prairie to urban, agriculture, or pasture affects aquatic systems via numerous pathways, including altered hydrology, water chemistry, sediment dynamics, temperature dynamics, channel geomorphology, trophic resources, riparian stability, and local habitat complexity.

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Collectively, the relations established between these pathways and associated biota illustrate the influence of anthropogenic activities on aquatic systems (Brown et al. 2005; Hughes et al. 2006).

Anthropogenic activities in watersheds are considered important in altering aquatic systems; however, our understanding of relations between human activities in a watershed and the constraints imposed by natural-occurring landscape factors on aquatic biota is extremely limited (Allan 2004). One problem in separating natural and anthropogenic effects is that the two often covary (e.g., soils and agriculture) (Allan 2004; King et al. 2005; Yates and Bailey 2010). When the natural conditions within a region are ignored, the effects of land use may be unintentionally overstated (Allan 2004). Wang et al. (2003) demonstrated that land use was a minor driver in the relatively undisturbed Northern Lakes region of central USA. Likewise, regions almost entirely dominated by a particular land use may not reveal a strong relation between land use and the abundance of organisms; however, natural factors may be more important when anthropogenic influences are minor (Allan 2004; Heitke et al. 2006).

Understanding land-use impacts on fishes is an essential component of fisheries management; however, separating the impacts of land use from natural landscape features is difficult because tools (i.e., geographic information system (GIS) coverages) must be available to separate land use from other environmental factors over large geographic areas to facilitate appropriate study designs. Smallmouth bass (*Micropterus dolomieu*) are a valuable recreational and ecological asset in many Missouri streams. Land-use changes over the past 100+ years in Missouri have resulted in substantial flow, temperature, and physical habitat changes in streams (Jacobson and Primm 1994), which have likely profoundly affected smallmouth bass populations (Fajen 1981).

We designed a study to investigate the covarying influences between natural landscape and in-channel features and dominant watershed land use on smallmouth bass populations in Missouri streams. A previous modeling study of smallmouth bass in Missouri streams (Brewer et al. 2007) identified two variables, the percentage of coarse-textured soils and soils with low runoff potential (hydrologic soil group D), as the most influential natural-occurring landscape features affecting the distribution and relative abundance of this species. Combinations of significant percentages of these important natural-occurring conditions were used to develop four groups with similar within-group characteristics, but dissimilar between-group characteristics, and then three land-use attributes were integrated into each group. The goal of the present study was to examine the hypothesis that the influence of watershed land-use conversion on populations of smallmouth bass is dependent upon the quality of natural watershed features. Objectives were to determine whether (*i*) densities of smallmouth bass were related to land-use attributes (forest, pasture, urban), natural-occurring watershed features (combinations of coarse-textured soils and runoff potential), or more importantly, the interaction between land-use attributes and natural-occurring features in the watershed and (*ii*) in-channel habitat features interacted with land use to determine differences in predicted densities of different age classes (young of year (YOY), age 1, and age >1) of smallmouth bass.

Materials and methods

Study area

Smallmouth bass occupy three biogeographic regions in Missouri: Central Dissected Till Plains, Osage Plains, and the Ozark Highlands (Nigh and Schroeder 2002). Study streams were located in each of these biogeographic regions (this encompasses the entire state, excluding the southeastern lowland areas). The Central Dissected Till Plains covers northern Missouri and is characterized by formerly glaciated plains that become increasingly dissected as they near major confluences, especially the Missouri and Mississippi rivers. Streams sampled in this region were limited to the northeastern section of Missouri, following the distributional limits of smallmouth bass (Brewer et al. 2007). The Osage Plains region occupies a large portion of west–central Missouri and is characterized by smooth plains with numerous bedrock outcroppings. Streams in the Central Dissected Till Plains and Osage Plains regions generally carry high sediment loads during periods of runoff and are low gradient and highly meandering; however, numerous sections of larger streams have been channelized. The Ozark Highlands region includes most of southern Missouri and is characterized by extensive geologic erosion, carbonate bedrock, and karst features. Many of the streams within this region are spring-fed and have low suspended sediment loads except during periods of high runoff. Historically, all of these regions were a mixture of prairie, savanna, woodland, and forest. Today, these regions have largely been converted to secondary growth forest, cropland, pasture, and urban areas, with small remnant tracks of native vegetation remaining (Nigh and Schroeder 2002). In this study, we classified stream segments from forested watersheds as "forest" and stream segments from grassland watersheds as "pasture".

Study design and site selection

Thirty-six stream segments (Fig. 1) were selected using criteria that allowed assessment of both natural and anthropogenic-induced variation in smallmouth bass abundance across Missouri. Criteria pertaining to natural variation in habitat conditions were based on watershed features having the strongest impact on smallmouth bass probability of occurrence and relative abundance (see Brewer et al. 2007 for a complete description). Briefly, statewide historical fish collections were spatially linked to natural-occurring environmental features (e.g., geology, soils, landform) using GIS, which was used as a basis for building probability-based models to predict presence and abundance of smallmouth bass in every stream segment in Missouri. Predicted abundances had good concordance with an additional, independent data set, and results indicated the fishery potential based only on natural features. Most of the variation in the predicted distribution and abundances of smallmouth bass could be explained using two natural-occurring conditions in the watershed: the percentage of coarse-textured soils and the percentage of hydrologic soil group D (indicative of soil permeability and runoff potential) (Brewer et al. 2007). Using varying amounts of these two environmental features (Table 1), multivariate cluster analyses of over 150 000 stream segments resulted in 18 distinct clusters (see Doisy et al. 2005 for a complete description). The four clusters chosen for this study (2, 3, 4, and 11; Table 1) contained the highest

Fig. 1. Map of Missouri, USA, showing the distribution of sampling sites, by group and land-use designation (pasture: triangles, forest: squares, and urban: circles). Groups correspond to the following clusters: Group A (Cluster 11), Group B (Cluster 3), Group C (Cluster 2), and Group D (Cluster 4).

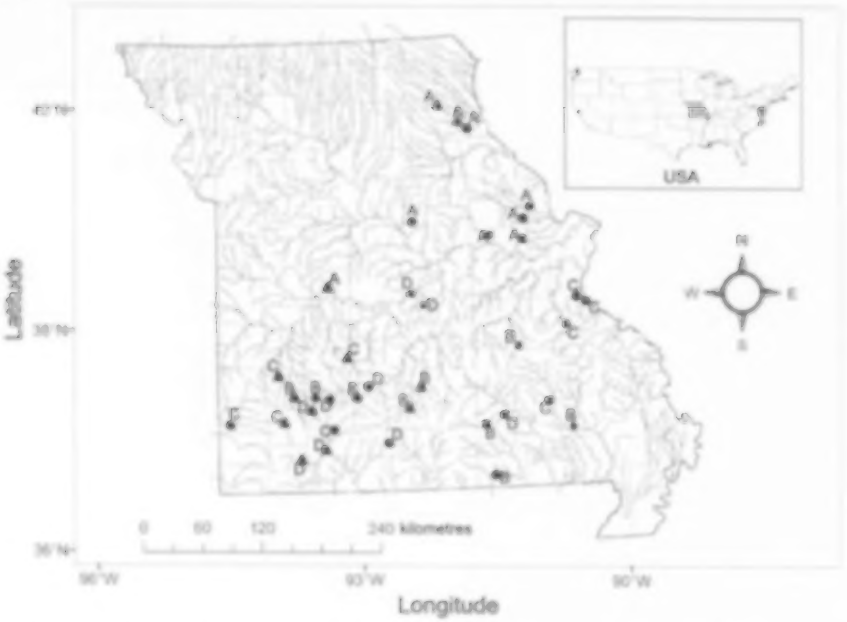


Table 1. Description of four groups (A–D) established based on modeling results from Brewer et al. (2007).

Group	Cluster	Natural conditions	Land-use designation	Forest (%)	Urban (%)	Pasture (%)
A	11	CTS < 50%, HSGD > 7%	Forest	62	<1	23
			Pasture	20	<1	46
			Urban	29	5	40
B	3	CTS > 90%	Forest	82	<1	18
			Pasture	33	<1	66
			Urban	31	2	66
C	2	CTS < 50%, HSGD < 7%	Forest	81	<1	18
			Pasture	16	<1	82
			Urban	29	13	53
D	4	CTS 50%–90%	Forest	62	<1	37
			Pasture	18	<1	81
			Urban	35	4	60

Note: Natural-occurring conditions in each group were assigned based on the percentages of coarse-textured soils (CTS) and hydrologic soil group D (HSGD) in the watershed. The mean percentage of each land use is provided for each land-use designation.

frequency of stream segments and therefore had the highest potential for achieving replication and interspersion. The clusters chosen were ranked based on the potential of population success. For this study, we equated population success with smallmouth bass abundance by assuming higher abundance indicated increased reproduction by adults or survival of young fish. The ranked clusters resulted in four groups (A–D), where Group A related to the lowest probability for population success and Group D related to the highest probability for population success. Groups B and C both had intermediate probabilities of population success, but were composed of different natural watershed conditions (Table 1).

Once groups were established, additional criteria were used to select stream segments within each group. Stream segments within each group were queried using GIS to identify segments that were perennial, had relatively similar drainage areas, and had a Shreve link of 10–250. Stream segments were then identified that had the highest percentage of forest, pasture, and urban use within their respective watershed, but were otherwise similar with regard to other human threats (i.e., mines, landfills). The area and percentages of each land use were determined using the land use and land cover data set created in the Missouri Gap Analysis Project (MoRAP 1998). Within this pool of potential sites, stream segments

were selected based on condition of riparian corridor (relatively intact) and, for access purposes, their proximity to public lands. Stream segments were assigned in each group to forest ($n = 3$), pasture ($n = 3$), or urban ($n = 3$) land uses based on the percentages of land use within each respective watershed. Attempts were made to intersperse forest, urban, and pasture sites (Fig. 1).

Habitat data

Landscape variables (i.e., watershed area, land-use attributes) (Table 2) were derived from existing geospatial data. We used a 1:100 000 digital stream network created by the Missouri Resource Assessment Partnership (Sowa et al. 2005). This coverage contains over 106 000 individual stream segments, each attributed with numerous landscape attributes. Land use was the variable of primary interest, but we included watershed area in case Shreve link was not sufficient to account for stream size.

Several in-channel variables (e.g., % pool) were measured on site at the time of fish sampling. The channel unit (CU) classification system proposed by Rabeni and Jacobson (1993) was simplified by using six CU types: pools, riffles, runs, vegetated edges, nonvegetated edges, and side-channel habitats (i.e., forewaters and backwaters). Each site was mapped prior to sampling so the relative percentages of CUs could be determined and flags or block nets could be placed at CU boundaries where necessary. Discharge was calculated using the velocity–area method (Gordon et al. 2004) at a minimum of two locations in each stream segment. Pool temperature (°C) was measured at mid-pool depth with a hand thermometer in a minimum of three pools or three pool sections (when pools were >200 m in length) at each site.

Remaining in-channel variables (e.g., sinuosity) (Table 2) were obtained from a previous study (Doisy et al. 2005), conducted on the same sites in summer 2005, using the US Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) protocol (Kaufmann et al. 1999). In-channel measures of sediment for the entire segment were based on measures from the sub-bank and embeddedness calculations of the EMAP protocol. Mean substrate size classes in all cross-sections were as follows: 1 = fines, 2 = sand, 2.5 = fine gravel, 3.5 = coarse gravel, 4 = cobble, 5 = small boulder, and 6 = large boulder. Specific variables were chosen from the EMAP data because we expected the variable to change because of land-use alterations (e.g., width to depth ratios) or the variables have been related to increased smallmouth bass densities or fitness (e.g., substrate, % fines) in other studies.

Fish sampling

Multiple methods of sampling were used because fish were collected over three biogeographic regions and under a variety of landscape conditions. Sampling methods used were snorkeling, above-water (AW) observation, and seining. Snorkeling and AW observation were combined to balance our ability to detect younger age classes and adults. A previous study examining the efficiency of these two methods indicated both sampling methods would be necessary to adequately characterize habitat use by multiple life stages of smallmouth bass (Brewer and Ellersieck, in press). Results indicated detection probabilities were significantly different among CUs when only one gear was used (i.e., comparisons of CU use would be biased when sampling shallow versus deeper-water CUs). Snorkeling was more efficient in deeper water, whereas AW observation was most efficient in shallow water. Run and vegetated edgewaters were the only CUs where there were no differences in detection probabilities depending on sampling method. Sampling using the two gears in appropriate CUs was generally comparable (probability of detection generally ranged between 0.5 and 0.7) and resulted in less fright bias among fish observed. Additionally, each method was found to be >90% accurate with respect to identification of YOY smallmouth bass (Brewer and Ellersieck, in press). Snorkeling was our primary gear (>60% of the time), but was not used if visibility to the stream bottom was <1 m. Seining was used in approximately 40% of the streams sampled and represented a small proportion of the stream segment (<20%). Seining was used in every group and land-use category, but most of the time (>40%) in stream segments classified by forest land use. We expect seining generally to be less efficient than direct observation (except where turbidity would prevent high efficiencies while observing; Fig. 2), but we also expect the distribution of methods across treatments to minimize bias or to underestimate fish densities in forest stream segments (where seining was most often used).

The choice of sampling method depended on CU sampled (i.e., related to detection probabilities from Brewer and Ellersieck, in press) and visibility. Snorkeling was the primary sampling method and was used when depths were >0.5 m in most CUs or 0.25 m in vegetated edges. AW observation was used when depths were <0.5 m if surface conditions did not impede observation. AW observation was used in edgewaters, shallow runs, and shallow pools. Only on one instance was snorkeling used to sample a shallow (<0.5 m) run because of high wind conditions that prevented us from being able to observe fish from above the surface of the water.

Fish sampling was conducted from July to September 2006. At each site, a minimum of 40 times channel width was sampled. Beginning at the most downstream point of the stream segment, CUs were sampled throughout the site so densities of each age class (YOY, age 1, or age >1) of smallmouth bass could be determined for the entire stream segment. Whereas riffles were mapped to calculate area, most were excluded from sampling because prior sampling indicated these CUs were used only very rarely in small streams by YOY smallmouth bass (Brewer 2008; Brewer and Ellersieck, in press). Nevertheless, 20 riffles were randomly selected and snorkeled, and no smallmouth bass were identified in any of them.

Each sampling gear had a specific protocol. When snorkeling was used, two observers began at the downstream end of the CU and moved upstream at a rate of approximately 5 m·min^{-1} to the end of the CU. Each snorkeler limited their observations to half of the stream and covered their half by swimming in a zig-zag pattern. Counted fish were limited to fish within approximately 2 m of the observer to increase the accuracy of viewing marked fish. The same approach was used for AW observations, only by walking slowly to minimize disturbance, through the CUs in a zig-zag pattern and verbally identifying fish counted with the other observer; however, one observer would walk along the bank while the

Table 2. Description (mean and standard deviation, SD) of habitat variables used in decision tree model development.

Group	Cluster	Variable	Mean	SD
A	11	**Land use (3)**	NA	NA
		Watershed area (km^2)	162	58
		Discharge ($m^3 \cdot s^{-1}$)	0.24	0.16
		Pool (%) in segment	86	21
		Temperature (°C)	19	4
		Channel sinuosity (m·m)	1.30	0.34
		Mean substrate size class (6)	3	0
		Fines (%)	11	7
		Riparian distance (m)	0	0
		Max. residual depth (cm)	132	47
		Mean width:depth (m·m)	38	11
B	3	**Land use (3)**	NA	NA
		Watershed area (km^2)	249	167
		Discharge ($m^3 \cdot s^{-1}$)	0.26	0.32
		Pool (%) in segment	71	25
		Temperature (°C)	26	4
		Channel sinuosity (m·m)	1.25	0.18
		Mean substrate size class (6)	3	0
		Fines (%)	3	2
		Riparian distance (m)	1	0
		Max. residual depth (cm)	150	88
		Mean width:depth (m·m)	39	11
C	2	**Land use (3)**	NA	NA
		Watershed area (km^2)	312	214
		Discharge ($m^3 \cdot s^{-1}$)	0.32	0.32
		Pool (%) in segment	59	25
		Temperature (°C)	26	4
		Channel sinuosity (m·m)	1.14	0.13
		Mean substrate size class (6)	3	0
		Fines (%)	4	4
		Riparian distance (m)	1	0
		Max. residual depth (cm)	101	49
		Mean width:depth (m·m)	32	8
D	4	**Land use (3)**	NA	NA
		Watershed area (km^2)	274	196
		Discharge ($m^3 \cdot s^{-1}$)	0.34	0.40
		Pool (%) in segment	65	16
		Temperature (°C)	26	2
		Channel sinuosity (m·m)	1.14	0.08
		Mean substrate size class (6)	3	0
		Fines (%)	6	4
		Riparian distance (m)	1	0
		Max. residual depth (cm)	106	38
		Mean width:depth (m·m)	39	9

Note: Bold font denotes ordinal variables, with the number of levels included in parentheses.

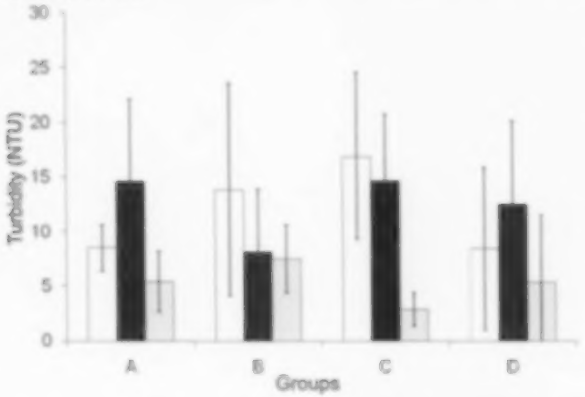
other walked streamside in edgewaters, and if water depth permitted (≥0.25 m), the streamside observer would snorkel slowly along the outside band of the water-willow, *Justicia americana*. Observers wore polarized sunglasses to minimize glare on the water's surface. When turbidity prohibited snorkeling or AW observation in any CU, a seine (4.57 m long and 1.22 m wide with 3 mm mesh) was used to carefully cover the same amount of area in an amount of time nearly equivalent to snorkeling. Seining began at the downstream end of the CU and continuous passes were made throughout the CU until all of the area within the CU was sampled.

Fish were identified on site and released downstream of the sampling point. YOY were readily distinguished from age-1 fish because of their smaller size and the presence of a tricolored tail. Age-1 fish did not have a tricolored tail and were noticeably smaller than the next age class. The sizes of fish encountered were in line with previous length-at-age studies in Missouri (Purkett 1958; Livingstone and Rabeni 1991; Reed and Rabeni 1989, and references therein).

Analyses

A two-way analysis of variance (ANOVA) was used to determine if differences existed in densities (YOY, age 1, and age >1) of smallmouth bass between the two factors —

Fig. 2. Mean turbidity (nephelometric turbidity units, NTU) ± 90% confidence intervals for streams in each land-use category (open bars = pasture, black bars = forest, and gray bars = urban land use) by group (A–D).



groups and land-use categories — and the interaction between the two factors ($\alpha \leq 0.10$). Data were rank-transformed because of failure to meet assumptions associated with ANOVA (i.e., normal distribution and equal variances). Rank transforming data results in loss of information, but approaches distribution-free nonparametric alternatives (Conover and Iman 1981). The test statistic for ranked data measures the difference between the weights of the groups instead of the differences in the original variables. The power of multiple comparisons procedures for non-normal populations is greater when rank-transformed data are used (Conover and Iman 1981). The Tukey–Kramer post hoc test was used to identify where significant differences ($\alpha \leq 0.10$) in smallmouth bass densities occurred between groups and land-use types. Data were analyzed using SAS (SAS Institute Inc. 2000).

Decision tree analyses were used to develop models to predict densities (number·m^{-2}) of YOY, age 1, and age >1 riverine smallmouth bass based on two landscape variables and a suite of stream segment variables (Table 2). The only two landscape variables included were watershed area and land use. Watershed area was included in case we did not adequately capture differences in stream sizes. Land use, on the other hand, was included as a structuring component to determine how in-channel habitat features might interact with land use to change the predicted densities of smallmouth bass. Decision trees are well suited to ecological data because they consider complex interactions (e.g., nonlinear) (Olden and Jackson 2002) and are able to identify a variety of conditions that may result in similar or completely different outcomes. Density of smallmouth bass in individual stream segments was weighted by the proportional abundance of each CU in that segment. Scatterplots were used to identify one outlier (Cowskin Creek). Prior to model building, this outlier was removed because densities were artificially inflated (nearly five times greater than any other stream) because of extremely low flows that reduced this stream to intermittent pools where fish were restricted. Answer Tree 3.0, an extension of SPSS statistical software package (SPSS Inc., Chicago, Illinois), was used to construct our decision tree models (significance was assessed at $\alpha \leq 0.10$). We used the Exhaustive CHAID algorithm to examine predictor variables to find the one that gave the best classification of the target variable by recursively splitting the data into subgroups until the analysis was completed based on our predefined stopping criteria. The Exhaustive CHAID algorithm was used because it does a more thorough job of examining all possible splits for each predictor variable and because it can produce more than two categories at any level in the tree (unlike C&RT and QUEST). Exhaustive CHAID was developed to improve the splitting capability by computing an adjusted p value for the predictor that provides the strongest association with the target variable.

The size of the tree (i.e., number of levels) that Answer Tree 3.0 allows is determined a priori by the user. The chosen stopping criteria reduces the probability of over-fitting the model. The minimum number of collections allowed in a child node was set to one, and the minimum number of collections allowed in a parent node was set to 10% of the total collections.

After the tree was developed, we manipulated our data by ±5% and developed new models using the same established criteria. Because our sample size was relatively small, we wanted to ensure the relations established between densities of smallmouth bass and habitat variables were strong enough to remain unchanged even if our data were manipulated to some degree.

Results

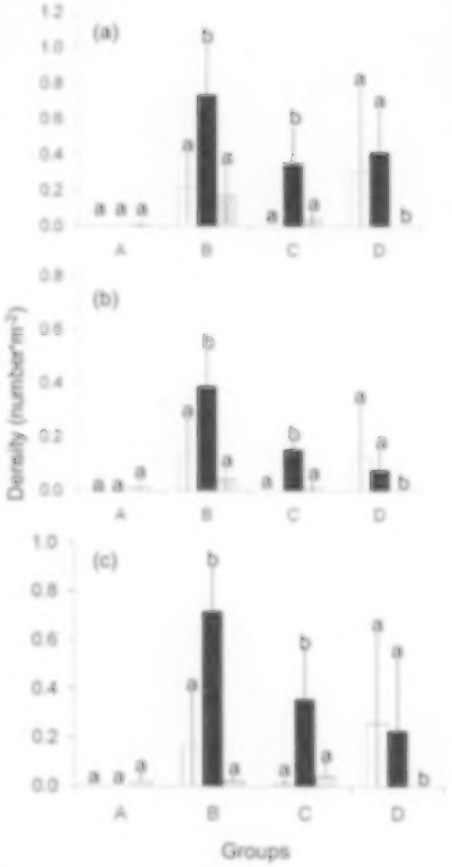
Land-use interactions with natural-occurring landscape features

The overall groups by land-use factorial ANOVA was significant ($F_{[11,34]} = 5.47$, $p = 0.0003$) for YOY smallmouth bass densities. Most importantly, the interactive effect of groups × land use was significant ($p = 0.03$), indicating the effects of land use depend on the natural conditions within the watershed (Fig. 3). As expected, Group A had the lowest densities of smallmouth bass, and this remained unchanged regardless of watershed land use. Groups B and C were the only categories of natural conditions where the amount of forest land use in the watershed related to higher densities of smallmouth bass. In Group D, urban streams had lower densities of smallmouth bass compared with the other land-use categories.

Factorial ANOVA for densities of age-1 smallmouth bass was significant ($F_{[11,34]} = 4.38$, $p = 0.001$). In this model, the interactive effect of groups × land use was also significant ($p = 0.06$; Fig. 3), indicating the effects of land use depend on the natural-occurring watershed conditions of coarse-textured soils and runoff potential. Again, Group A generally had the lowest densities of smallmouth bass, regardless of land use. The highest densities of smallmouth bass in Groups B and C were in forest stream segments. In Group D, urban streams had lower densities of smallmouth bass compared with the other land-use categories.

Factorial ANOVA for densities of age >1 smallmouth bass was significant ($F_{[11,34]} = 4.50$, $p = 0.001$). Like models created for other age classes, the interactive effect of groups × land use was significant ($p = 0.03$; Fig. 3). The pattern was similar to the other age classes, with Group A having the

Fig. 3. Mean density (number·m^{-2}) of different age classes (panel (*a*): young of year, panel (*b*): age-1, and panel (*c*): age >1) of smallmouth bass (±90% confidence intervals) by group (A–D) and land-use designation (open bars = pasture, black bars = forest, and gray bars = urban land use). Different letters indicate significant differences between land-use types within each group (based on rank-transformed values).



lowest densities of fish, regardless of land use in the watershed, Groups B and C having the highest densities of fish in forested stream segments compared with other land-use categories, and Group D with no detectable differences between densities of fish in forest and pasture stream segments, but with significantly lower densities in urban stream segments.

Viewing the models by groups, several patterns emerge. First, Group A, predicted to have the lowest potential for population success based on natural landscape factors in the watershed, had the lowest densities of fish, regardless of age class or land use in the watershed. Second, Groups B and C, predicted to have moderate but similar population success, both had higher densities of smallmouth bass in forested stream segments compared with stream segments in other land-use categories, regardless of age class. Finally, Group D, predicted to have high population success based on natural conditions in the watershed, had higher fish densities in forest and pasture stream segments when compared with urban streams.

Predicting densities of smallmouth bass using land use and in-channel features

Young of year

The most important variable ($F_{[2,32]} = 4.39$, $p = 0.02$) in our model predicting densities of YOY smallmouth bass was land use (Fig. 4). Densities of YOY were predicted to be nearly threefold higher in stream segments located in forested watersheds compared with those in watersheds classified by pasture and sixfold higher than those stream segments classified by urban land use. Land use explained 23% of the variation in our data. Densities were predicted to more than double in larger stream segments (discharge > 0.47 m^3·s^{-1}) located in forested watersheds. Discharge ($F_{[1,10]} = 16.35$, $p = 0.04$) accounted for an additional 21% of variation in our data. Water temperature was significant ($F_{[1,7]} = 12.96$, $p = 0.08$) in relatively smaller streams (discharge ≤ 0.47 m^3·s^{-1}), with warmer water temperatures (>21 °C) nearly doubling predicted densities of YOY. Overall, our model explained 62% of the variation in YOY densities. The same variables were significant to our decision tree after manipulating our data by ±5% and creating new models using the same habitat variables.

Age 1

The only variable significant in our model predicting densities of age-1 smallmouth bass was land use ($F_{[2,32]} = 3.27$, $p = 0.05$). Mean densities of age-1 fish were greatest in stream segments located in forested watersheds, followed by pasture and urban watersheds, respectively (Fig. 5). This model explained only 17% of the variation in our data. Land use remained the only significant variable in our model after manipulating densities by 5%.

Age >1

The most important variable ($F_{[2,32]} = 4.92$, $p = 0.01$) in our model predicting densities of age >1 smallmouth bass was land use (Fig. 6). Mean densities of age >1 fish were predicted to be threefold higher in stream segments located in forested watersheds than those in watersheds classified by pasture and more than 15-fold higher than those stream segments located in watersheds classified by an urban landscape. Land use explained 23% of the variation in our data. Mean densities of smallmouth bass were predicted to more than double in larger stream segments (discharge > 0.24 m^3·s^{-1}). Discharge ($F_{[1,10]} = 23.15$, $p = 0.01$) accounted for an additional 37% of variation in our data. The percentage of fines in the channel was significant ($F_{[2,9]} = 51.33$, $p = 0.0003$) in stream segments located in watersheds classified by pasture land use. Predicted mean densities of age >1 fish increased approximately fivefold when percent fines ranged between 1.9% and 3.6%; however, when the percentage of fines present in the channel was greater than or less than this range, mean densities of smallmouth bass were predicted to be much lower (but the response was similar in either direction; Fig. 6). Overall, our model explained 81% of the variation in age >1 densities. The same variables were significant to our decision tree after manipulating our data by 5% and creating new models using the same variables.

Fig. 4. Decision tree model indicating predicted densities (number·m^{-2}) of young-of-year smallmouth bass based on land use and in-channel variables. The predicted density (bold numbers) and sample size (in parentheses) are shown for the entire data set and at each node. Child nodes are represented by circles.

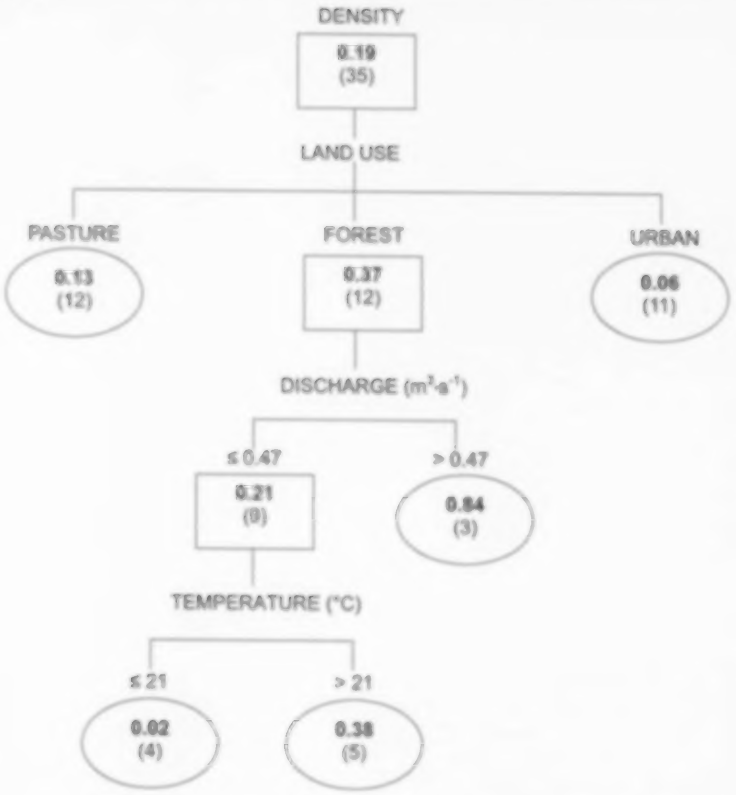
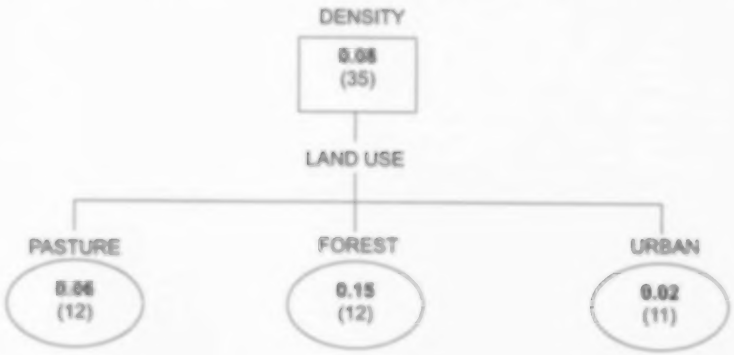


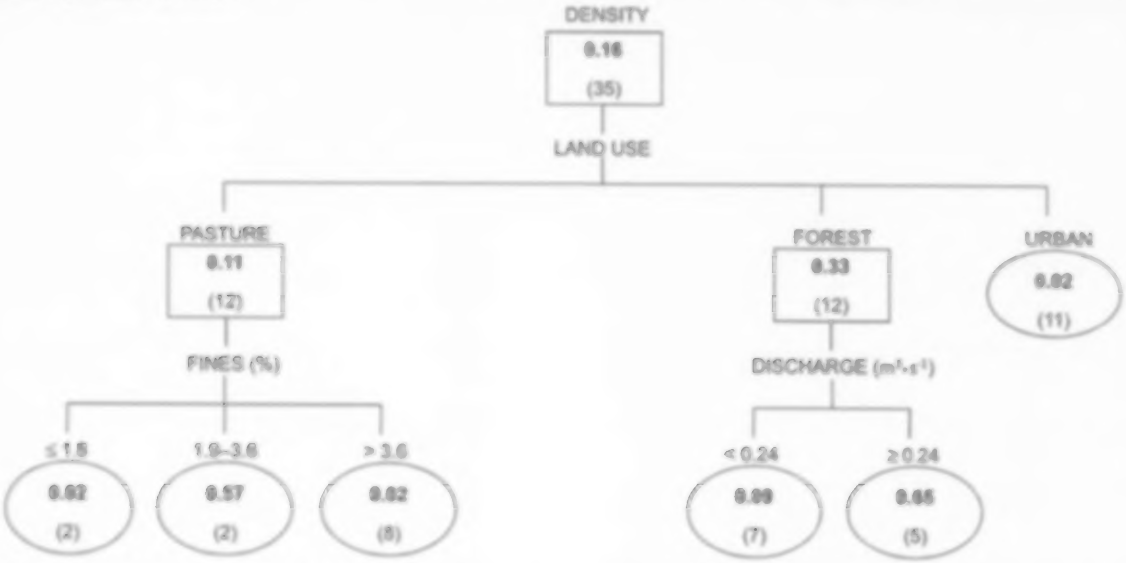
Fig. 5. Decision tree model showing the predicted densities (number·m^{-2}) of age-1 smallmouth bass based on land use and in-channel variables. The predicted density (bold numbers) and sample size (in parentheses) are shown for the entire data set and at each node. Child nodes are represented by circles.



Discussion

The natural watershed conditions most related to distributions and densities of smallmouth bass in midwestern and southern United States' streams have been coarse-textured or rocky soils or substrates: Iowa (Paragamian 1981), Alabama (Hubert 1981), Michigan (Rankin 1986), Wisconsin (Lyons 1991), Missouri (Pflieger 1997), and Arkansas (Hafs et al. 2010). In a previous study (Brewer et al. 2007), we predicted the probability of smallmouth bass occurrence to nearly double when >57% coarse-textured soils were in the watershed; it appears mean densities also approximately doubled. Therefore, the initial clustering of streams in this study was appropriate and resulted in a gradient of poor (but adequate) to very good natural conditions for this species and provided the appropriate backdrop for examining effects of land use under different natural conditions.

Fig. 6. Decision tree model showing the predicted densities (number·m^{-2}) of age > 1 smallmouth bass based on land use and in-channel variables. The predicted density (bold numbers) and sample size (in parentheses) are shown for the entire data set and at each node. Child nodes are represented by circles.



The relation between land use and densities of smallmouth bass varied depending on the natural conditions used to define each group. The low densities, with little relation to land use, in Group A may be a result of the natural conditions (low percentage of rocky soils and high runoff potential); increasing the percentage of forest does little to improve conditions for smallmouth bass. Also, "legacy effects" (Allan 2004) from historical natural or anthropogenic disturbances (Harding et al. 1998) may inhibit smallmouth bass fitness in these regions where channel alterations may exist with an unknown recovery time. These sites were not geographically clustered; however, these sites and the conditions within this group appear to follow the distributional limits of smallmouth bass in Missouri (see Brewer et al. 2007). Groups B and C (predicted to have moderate population success based on natural conditions), on the other hand, have appreciable densities of smallmouth bass in forested watersheds, but land-use attributes other than forest appear to have strong negative effects on smallmouth bass abundance. Urban streams, regardless of groups, generally had the lowest densities of fish. Pasture conditions, on the other hand, appear to impact streams in Groups B and C more so than in Group D (where there were no differences in densities between forested and pasture streams). We interpreted this as support for our hypothesis that natural conditions in Groups B and C would allow for moderate population success by smallmouth bass; thus, pasture influences largely decrease the potential of a smallmouth bass recreational fishery within these regions. Group D showed no difference in densities between forest and pasture streams, indicating the conditions in these watersheds are relatively resilient to pasture land use, or the type of pasture use in these areas ameliorates some of the negative impacts of this activity (see Marshall et al. 2008). It appears that the better the natural conditions are in the watershed for smallmouth bass populations, the more resilient these populations will be to land-use changes, realizing that a threshold will likely exist where this will no longer be the case. This threshold, although unknown, is expected to be different because of the natural conditions that occur within each watershed. For example, the mean percentages of watershed in pasture land use is 82% and 81% in Groups C and D, respectively, but these similar percentages relate to significantly lower fish densities in Group C, but not Group D.

Forested watersheds generally had the highest densities of smallmouth bass, with or without the influence of natural watershed features. Land use was the most significant variable in our decision tree models for each age class, with forested watersheds generally having the highest densities of smallmouth bass. Other researchers have found a positive relation between forest cover and fish-assemblage structure and densities (Steedman 1988; Maret et al. 1997), whereas urbanization (Weaver and Garman 1994; Onorato et al. 2000; Walters et al. 2005) and to a lesser extent, pasture (Meador and Goldstein 2003) result in degraded community structure. Streams classified by urban and pasture land uses had lower densities of smallmouth bass in most groups (the only exception was pasture land use in Group D). Urban streams had lower densities of smallmouth bass than streams classified by other land-use categories, indicating a disproportionate effect when considering the amount of urban land use in each watershed (the maximum amount of urban land use in any watershed was 22%, but the mean of all urban streams was only 6%). Other researchers have identified significant changes in biota in watersheds with >10% urban influence (see Paul and Meyer 2001 for a review). Mean densities of fish in streams classified by pasture land use were not significantly different than urban stream densities, except in Group D. It is difficult to make comparisons between these findings and those of others regarding the influence of pas-

ture land use, as many have considered pasture part of agriculture, whereas we distinctly separated pasture from row crops. Meador and Goldstein (2003) argue that some catchments with high percentages of agriculture associated with relatively good fish community condition may respond to other factors within the region or management practices that reduce physicochemical degradation in streams. Marshall et al. (2008) further dissected pasture land use into Conservation Reserve Program (CRP) lands to show positive changes in species richness in coldwater streams related to CRP practices. These studies collectively indicate best management practices within land-use categories should be considered when examining impacts to stream biota. In addition, the present study indicates some of the discrepancies among studies may relate to underlying natural-occurring landscape features and may only be slightly improved by having appropriate in-channel habitat features.

Land use was the only variable significant in our age-1 model, whereas several in-channel features (discharge, % fines, and temperature) contributed to increases or decreases in densities of other age classes. In our YOY and age >1 models, higher discharge in stream segments in forested watersheds related to less than or equal to a fourfold increase in fish densities. Smallmouth bass are expected to increase in abundance as stream size increases (Zorn et al. 2002; Brewer et al. 2007; Dauwalter et al. 2007) to an unknown threshold where habitat may become less suitable. Generally, hydraulic variation is lower in larger streams and habitat characteristics are more stable. Highly variable flows in smaller streams affect reproductive success (Orth and Newcomb 2002), and a lack of deeper-water habitats and other abiotic factors (e.g., temperature; Coutant 1975) may result in marginal conditions for adult piscivores to thrive in smaller streams (Schlosser 1987). It appears this relationship is also applicable for YOY; however, as anticipated by the optimum temperature (29 °C) for YOY growth (Shuter and Post 1990), higher temperatures in smaller streams were important to YOY. Higher temperature selection by YOY smallmouth bass has also been shown in a field-based study on Missouri streams (Brewer 2008). In watersheds classified by pasture land use, moderate percentages of fines related to low densities of smallmouth bass. Excess fine sediments likely provide unsuitable habitat for important macroinvertebrate prey of smallmouth bass. Sedimentation due to agricultural (Lenat 1984) and range (Meador and Goldstein 2003) land-use practices is indicated as a factor related to fish population declines; likely, pasture land use has a similar impact. However, macroinvertebrate fauna may be enhanced with increasing amounts of pasture land use until some threshold is reached (Quinn and Hickey 1990; Quinn 2000), which may explain why densities of smallmouth bass increased with a slight increase in fines and then declined as the percentage of fines in the channel continued to increase. There are other finer-scale habitat features that might be important to how YOY are distributed in streams, and these factors change depending on dominant land use in the watershed (Brewer, in press).

For conservation or restoration activities to be effective, a comprehensive understanding of the interactive effects between natural-occurring environmental conditions and anthropogenic influences is needed. Here, changes in smallmouth bass densities that occur with different combinations of land-use activities have been identified in a variety of natural settings at two spatial scales (watershed and in-channel). To further address this issue, studies are needed that will examine the specific mechanisms that promote these changes and how improvements in the landscape (usually defined as best management practices) affect these populations or if alternative strategies are necessary. Nonetheless, the information presented here should help guide priorities for policies relating to such questions as whether to protect vulnerable watersheds, or only the most resilient, or what activities to allow or encourage in watersheds with different vulnerabilities. For example, it is unlikely that increasing the percentage of forest area in Group A will improve the smallmouth bass fishery in this region. We argue that Group A, signified primarily by high runoff potential, has naturally flashy stream conditions that will not be mitigated to improve the fishery by increasing the percentage of forested habitat. In addition, this study indicates that some in-channel factors may increase the potential for population success when considering dominant land-use characteristics in the watershed. This information, when known, is helpful in identifying management areas with greater fishery potential associated with particular land uses. For example, in pasture-dominated watersheds, management actions controlling excess in-channel sedimentation may increase population numbers (presumably related to increased fitness). The conditions identified in this study occur outside of the state of Missouri and likely have the same implications for smallmouth bass conservation and management. Understanding the geomorphic connections between land-use attributes within natural settings and channel morphology may provide additional insight to the changes in biota occurring within these regions. Realizing the true connection between anthropogenic and natural settings is necessary to increase the effectiveness of conservation and management practices.

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Atlantic bluefin tuna (*Thunnus thynnus*): a state-dependent energy allocation model for growth, maturation, and reproductive investment

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Abstract: The relationship between Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) life history patterns and environmental conditions was investigated by developing a state-dependent model that optimizes energy allocation between growth and energy stores and the decision to spawn. The model successfully recreates growth, age-at-maturity, and seasonal variability in condition for western ABFT that spawn primarily in the Gulf of Mexico. Eastern ABFT spawning in the Mediterranean Sea display a life history trajectory shifted toward earlier maturation and, perhaps, reduced growth — a pattern predicted by the model when mortality was higher, migration distance shorter, and food intake during migration and spawning higher. Simulations highlight the sensitivity of the optimal ABFT life history strategy to variability in net energy intake, particularly during migration and spawning, a poorly understood component of their life cycle. Results also emphasize the importance for optimal life history patterns of the timing of spawning migrations in relation to the phenology and amplitude of seasonal prey availability. This study provides insight into potential mechanisms that underlie observations that are at the heart of current discussions regarding ABFT subpopulation structure and variable life history patterns.

Résumé : La mise au point d'un modèle dépendant de l'état qui optimise l'allocation d'énergie entre la croissance et les réserves énergétiques et la décision de frayer a permis d'étudier la relation entre les patrons du cycle biologique du thon rouge (ABFT, *Thunnus thynnus*) et les conditions de l'environnement. Le modèle représente avec succès la croissance, l'âge à la maturité et la variabilité saisonnière de la condition chez les ABFT de l'ouest qui fraient principalement dans le golfe du Mexique. Les ABFT de l'est qui fraient dans la Méditerranée présentent une trajectoire de cycle biologique avec un déplacement vers une maturation plus hâtive et, peut-être, une croissance réduite — c'est un patron prédit par le modèle quand la mortalité est plus élevée, la distance de migration plus courte et l'ingestion de nourriture durant la migration et la fraie plus grande. Les simulations soulignent la sensibilité de la stratégie optimale du cycle biologique des ABFT à la variabilité de l'ingestion nette d'énergie, particulièrement durant la migration et la fraie, une composante mal comprise de leur cycle biologique. Les résultats soulignent aussi l'importance pour l'obtention de patrons optimaux du cycle biologique d'un calendrier des migrations de fraie en relation avec la phénologie et l'amplitude de la disponibilité saisonnière des proies. Notre étude ouvre une perspective sur les mécanismes potentiels qui sous-tendent les observations qui alimentent les discussions actuelles sur la structure des sous-populations et les patrons variables du cycle biologique des ABFT.

[Traduit par la Rédaction]

Introduction

In speed, grace, and hunting effectiveness, tuna are virtually unmatched among marine top predators. The elusive, nomadic lifestyle of tuna species makes them a challenge for scientists, managers, and fishermen — a fact that may be responsible for the continued persistence of this species despite centuries of exploitation. However, in recent decades Atlantic bluefin tuna (ABFT, *Thunnus thynnus*), a commercially valuable migratory tuna with a basin-scale distribution, have become the focus of increasing fishing pressure (ICCAT 2009), raising questions concerning the fish's sustainability under current management practices (ICCAT 2009; Fish and Wildlife Service 2009). The International Commission for the Conservation of Atlantic Tunas (ICCAT) is responsible for managing ABFT, which it delineates as a western (W-BFT) and an eastern (E-BFT) stock. ICCAT's two-stock management approach is supported by differences in life history characteristics (E-BFT mature smaller and earlier than W-BFT), and there appears to be regional natal homing to the spawning grounds in the Mediterranean Sea and Gulf of Mexico, respectively (Secor 2002; Rooker et al. 2008).

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Life history trajectories can reflect the environment a fish population experiences and has adapted to, though the reasons for observed differences in life history between the stocks are not understood. For example, E-BFT migration distance appears to be shorter (although this assumption deserves further study), and these fish have experienced more intense fishing pressure for a longer time than W-BFT (Fromentin and Powers 2005).

Overall, a great deal of uncertainty remains regarding basic life history traits and population substructure for this species, particularly for W-BFT (Fromentin and Powers 2005; Rooker et al. 2007; Galuardi et al. 2010). For example, the presence of presumably mature fish outside the known spawning areas during the breeding period (Lutcavage et al. 1999; Block et al. 2005; Galuardi et al. 2010) suggests that either unknown spawning areas are available to ABFT or skipped spawning is a common behavior for this species. Some of this uncertain information forms the basis of calculations of ABFT stock productivity forecasts, rebuilding potential, and threshold fishing mortality that are key components of the current ICCAT management approach (ICCAT 2007; Secor 2007). Addressing these uncertainties and improving our understanding of the interplay between life history traits and population structure is important for improving the effectiveness of ABFT management (Rooker et al. 2007; ICCAT 2009).

Life history models can be useful tools for exploring the theoretical interplay among ecosystem conditions and emerging life history traits, spawning behavior, and seasonal variability in condition resulting from allocation strategies for fish species (Jørgensen and Fiksen 2006; Jørgensen et al. 2006, 2008). Our primary objective is to develop such a model that recreates observed patterns in population characteristics (growth, age-at-maturity, seasonal changes in condition) for ABFT. By exploring the interplay between environmental conditions and ABFT population characteristics, we obtain insight into potential mechanisms responsible for observed differences between E-BFT and W-BFT and examine whether skipped spawning would be an optimal spawning behavior for this species.

To meet these objectives, we developed a state-dependent model of growth, maturity, and energy allocation to reproduction. In this model, ABFT growth, condition (length–mass relationship), age-at-maturity, and size-at-age relationships emerge from the interaction among environmental conditions and the individual energy allocation and spawning strategy that is optimal in that environment. Simulations were selected to explore the influence of key environmental variables that may differ between ABFT subpopulations, which include distance between feeding and spawning areas, food ingestion during migration and spawning, and an annual mortality rate that integrates natural and fishing mortality.

We have included functional relationships from theory or other species where ABFT biology is scarce. For example, hydrodynamic costs of swimming have not been quantified for tuna of different condition factors, but hydrodynamics predicts that drag increases with cross-sectional area (Vogel 1994). We've implemented increasing swimming costs with increasing condition. With spawning costs, there is a fundamental difference between the energetic costs of getting to and from the spawning grounds and the timing of the migration, which can imply opportunity costs (or benefits). For example, foraging opportunities can be lost if an individual must leave the feeding area to migrate when feeding conditions are still good. Alternatively, foraging opportunities can be gained when individuals leave suboptimal feeding areas for better ones. Consequently, timing of migration incurs an added benefit or cost, as it determines the location of individuals in relation to seasonal food availability throughout a species' range (e.g., Varpe et al. 2007). Compare this with swimming costs, which are often less for smaller fish, thus involving energy units rather than time, and where functions and trade-offs are more likely to be linked to size. In our approach, we've modeled the costs of migration that includes both the costs and benefits associated with the timing and energetic costs of migration. A model for the purposes of this study that would include more specific processes (e.g., vertical migration behavior and thermoregulation) is not possible given the complex life history and current biological understanding of ABFT.

Our methodological direction aligns with mechanistically rich modeling (DeAngelis and Mooij 2003) and combines species-specific relationships, when known, with general relationships. This approach can reveal where our knowledge is sound, where critical gaps exist, and which parameters are most important to model behavior.

Materials and methods

Model overview

At monthly time steps, ABFT (*i*) feed, (*ii*) optimally allocate surplus energy to growth or to storage, and (*iii*) when optimal, either migrate to and from a spawning site where they use their stored energy to develop gonads or remain in the feeding area and continue to allocate energy to growth or storage. In addition to energy allocation and the decision to migrate and spawn, the level of stores that remain after spawning is also optimized. This cycle is repeated up to an effective maximum lifespan of 25 years. In reality, fish may live beyond 25 years, but the maximum age in the model represents an effective maximum age in terms of population-level reproductive effort. The optimization criterion is expected lifetime energy available for egg production, and the model only considers females. Energy allocation is state-dependent, with states being age (in months), body length (in cm), stored energy (proportion of full), and current level of food availability (needed because of the autocorrelated feeding environment).

Energy balance is calculated in different ways during time spent on the feeding area, during migration, and during spawning. ABFT make extensive vertical migrations each day to different environmental regimes (Lutcavage et al. 1999; Block et al. 2001; Wilson et al. 2005), presumably to maximize access to prey and to minimize stresses imposed by vertical distributions of temperature, oxygen, and predators. An additional important consideration is that large ABFT are warm-bodied (e.g., Carey and Lawson 1973; Lawson et al. 2010) and thus unique in comparison with most other pelagic fishes. In terms of their thermal and cardiorespiratory physiology, large size, heat retention systems, and other biochemical attributes confer distinct (but of course limited) advantages and allow ABFT to surpass thermal constraints encountered by most fishes, even of similar sizes.

Their body temperatures in the cool feeding grounds are actually 10–15 °C higher than ambient (Lawson et al. 2010). In this respect they're functional homeotherms, and while temperature and oxygen are certainly important factors determining ABFT behavior, it is assumed that these processes are most important at a smaller spatio-temporal scale than is the focus here.

For these reasons, during time spent in the feeding area (prior to maturity and otherwise when not either migrating or spawning), energy balance is assumed to reflect a seasonally varying and stochastic food environment. The modeled food environment during this time has both seasonality and autocorrelated stochasticity to reflect typical temporal patterns in prey availability and thus tuna foraging efficiency. During migration, energy balance reflects the energetic cost of movement (a function of the physical costs of movement and time spent away from potentially more productive feeding areas) and is modified by opportunistic feeding. During spawning, energy balance is a function of an estimated activity cost of spawning and again is modified by some opportunistic feeding.

The growth environment experienced by individual cohorts in the model varies according to the stochasticity imposed on the food environment of the feeding area. Thus, model results averaged over a long time period (thousands of years) allow several population characteristics and their variability to emerge, including growth trajectories, maturation ages and sizes, reproductive investment strategies, and population dynamics, including age and size structure.

The model used in this study is based on a similar approach for the Atlantic cod (*Gadus morhua*; Jørgensen and Fiksen 2006). The bioenergetics framework is built on the Wisconsin bioenergetics model (Hewett and Johnson 1992) but modified to fit parameters and relationships available for ABFT. Alternative frameworks for bioenergetics modeling include dynamic energy budgets (DEB; Kooijman 2000) and the metabolic theory of ecology (MTE, Brown et al. 2004). MTE is designed to be general and does not easily allow incorporation of species-specific parameters or relationships, but the reliance on allometric scaling resembles our approach. DEB allows easier incorporation of species detail, but contains assumptions that constrain the type of output a model can produce. For example, DEB assumes that allocation to reproduction happens over the entire lifetime, which might be appropriate for some organisms but less so for long-lived fish. In contrast, our approach is designed to study evolution of maturation and reproduction as flexible traits that emerge from evolutionary considerations. Fitness maximization combines a backwards iterating algorithm that produces an optimized life history plan (Houston and McNamara 1999; Clark and Mangel 2000) and a forward simulation that allows visualization of individual and population characteristics emerging from the interaction between the life history plan and environment. The proposed model is intended to be a generalized model that is not specified for either W-BFT or E-BFT, though the reference scenario is designed to represent conditions experienced by W-BFT.

Individual physiology

The individual body mass is compartmentalized into irreversible, structural mass and reversible, energy storage (Roff 1983). Structural mass ($W_{\text{Structure}}$, g) is a function of length and is calculated as

$$(1) \qquad W_{\text{Structure}}(L) = \frac{K_{\text{Min}} \cdot L^3}{100}$$

where K_{Min} is the minimum Fulton's condition factor (unitless) and L is the fish body length (cm), which we define as the straight fork length (cm).

wAdditional energy (E_{Stores}, kJ) above $W_{\text{Structure}}$ can be stored to meet energy demands of activity (migration, searching for prey), spawning (egg production, in the case of females), and starvation in periods of low food availability. ABFT primarily store energy as intramuscular lipid stores and proteins embedded in muscles, and prior to spawning additional lipids are stored as perigonadal fat. Here, energy stores are defined as the combined intramuscular and perigonadal fat stores and increased protein stored in the muscles. The mass in energy stores (W_{Stores}, g) is calculated as

$$(2) \qquad W_{\text{Stores}}(E_{\text{Stores}}) = \frac{E_{\text{Stores}}}{\rho_{\text{Stores}}}$$

where ρ_{Stores} (kJ·g^{-1}) is the wet-mass energy density of stores.

The total mass of the tuna ($W_{\text{Total}}(L, E_{\text{Stores}})$, g) can then be calculated as

$$(3) \qquad W_{\text{Total}}(L, E_{\text{Stores}}) = W_{\text{Structure}}(L) + W_{\text{Stores}}(E_{\text{Stores}})$$

A second parameter, K_{Max}, sets the maximum amount of energy stores that can be carried by an individual fish ($E_{\text{MaxStores}}$, kJ):

$$(4) \qquad E_{\text{MaxStores}}(L) = \rho_{\text{Stores}}(K_{\text{Max}} - K_{\text{Min}})\frac{L^3}{100}$$

The values for K_{Min} and K_{Max} were estimated based on an analysis of length and mass data for 4830 ABFT between 185 and 305 cm (8- to 25+-year-old fish; Turner and Restrepo 1994) from commercial fishery landings in the Gulf of Maine region. These data were collected across the period when ABFT condition is expected to be at a minimum (June, following spawning) and at a maximum (November, at departure from primary feeding area). The values for K_{Min} and K_{Max} were set at two standard deviations from the mean Fulton's K value for the length and mass data set.

Energy budget overview

A net intake function for a fish carrying no energy stores, modified by seasonal and stochastic variability in the food environment, is used to calculate the amount of energy ingested by an individual fish. The intake function is further modified by subtracting the cost of carrying energy stores (lipids), which is a metabolic cost assumed to be less than that for carrying somatic tissue. If a positive amount of energy remains after the cost of carrying stores is subtracted from the intake function, energy is ingested and a variable proportion of energy is allocated toward either growth (increasing length and structural mass) or energy stores (increasing mass of stored lipids and proteins). If a negative energy value remains, the energy deficit is drawn from energy stores.

Energy intake

A single function for net energy intake is used. Net energy intake is the difference between ingested energy available for production and all metabolic costs. As such, the net intake function integrates variability in prey availability, prey quality, and metabolic costs over time and accounts for digestive inefficiency. This approach minimizes the potential for compounding uncertainties associated with an estimation of each of the integrated processes.

The average net energy intake (I, kJ·month^{-1}) of a lean tuna is assumed to be an allometric function of fish length:

$$(5) \qquad I(L) = a \cdot L^b$$

The coefficient a (kJ·cm^{-b}·month^{-1}) is set to match observed growth for the species, and the exponent b (unitless) is derived from published estimates for the scaling of ingestion and metabolic costs with ABFT mass (Overholtz 2006; Fitzgibbon et al. 2008).

To introduce individual variability in the model, the average intake I is modified by two components of environmental variability (seasonality and temporally autocorrelated stochasticity) and one component of individual variability (the metabolic cost of the individual's stores, C_{Stores}):

$$(6) \qquad I_{\text{Net}}(L, E_{\text{Stores}}, t) = I(L) \cdot [S(t) + X(t)] - C_{\text{Stores}}(E_{\text{Stores}})$$

Here $S(t)$ is seasonal variability in prey availability and quality, and stochasticity $X(t)$ represents the inherent background variability in the food environment.

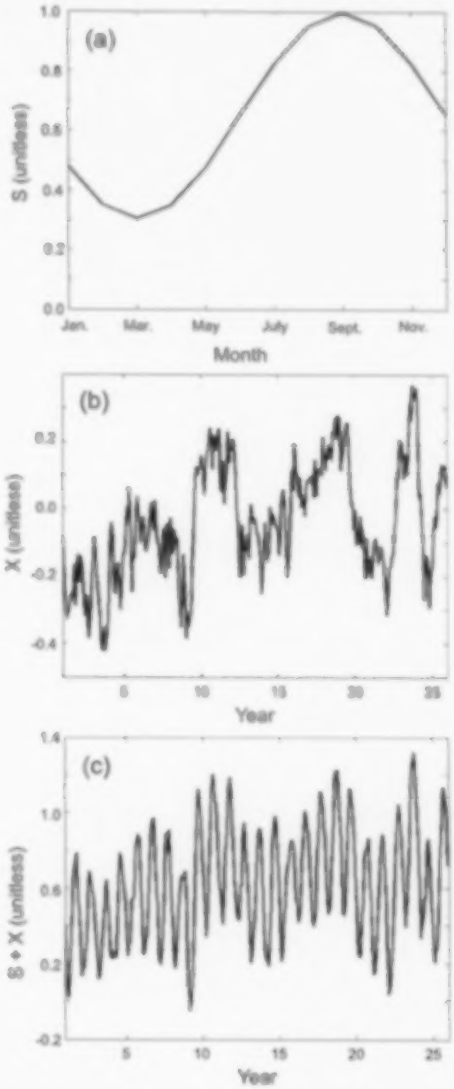
Seasonality

The majority of annual growth in length for juvenile ABFT occurs between June and November in both the eastern and western Atlantic (Mather and Shuck 1960; Furnestin and Dardignac 1961; Cort 1991). This suggests that ABFT experience strong seasonality in net intake as a consequence of annual cycles in environmental components that influence the difference between energetic intake and metabolic costs. Presumably, the seasonality in net intake reflects variability in photoperiod, prey aggregation, and prey quality in their primary feeding areas. These processes are integrated in a seasonality term that is set initially as a sine function of day-of-year ($S(d)$) that is characterized by an amplitude (S_{Amp}, unitless) and mean (S_{Mean}, unitless) value and a day that the seasonal peak intake occurs (D_{Peak}, day of year) (Fig. 1*a*):

$$(7) \qquad S(d) = S_{\text{Mean}} + \sin\left[\frac{d + \pi(90 - D_{\text{Peak}})}{360}\right] \cdot S_{\text{Amp}}$$

A sine function was selected because seasonality is expected to approximate seasonal variability in photoperiod, which approximates a sine curve over an annual cycle. Monthly mean values are calculated from the daily seasonality function as required by the monthly model time step. Calculating seasonality on a daily time scale allows for peak seasonality to be varied by time increments of less than a month. The initial setting for the seasonal peak is set as 15 August based on an estimated time when ABFT are gaining mass at the greatest rate in higher-latitude feeding areas (W. Golet and M. Lutcavage, unpublished data; Mather and Shuck 1960).

Fig. 1. Variability in the value for annual seasonality (*a*) and a 25-year example of variability in environmental stochasticity (*b*) and the environment conditions (combined seasonality and environmental stochasticity) (*c*) that determine net energy intake in the feeding area.



Stochasticity

Food intake in the model is also modified by a term that accounts for temporally autocorrelated environmental variability ($X(t)$). This variable is based on the following equation that includes terms that set the extent of autocorrelation (C_1), inherent variability (C_2), and the amplitude of the variability (C_3) in the system:

$$(8) \qquad X(t) = \overline{X} + \left\{ C_1 \cdot [X(t-1) - \overline{X}] + C_2 \cdot N \cdot \sqrt{1 - C_1^2} \right\} \cdot C_3$$

Here, $\overline{X}$ is the average value of the coefficient (chosen to be 0), and N is a random number drawn from a standard normal distribution $N(0,1)$. As an example, a 25-year period of the stochasticity term and the combined seasonality and stochasticity term is provided (Figs. 1*b*–1*c*). The initial setting for the stochasticity parameters creates low-frequency interannual variation such that unusually good or bad feeding conditions may persist for multiple years.

Cost of carrying energy stores

The model includes two sources of energetic costs required to maintain energy stores: the metabolic costs of maintaining store tissue and the influence of a change in body shape on the energy required for movement (hydrodynamics). This metabolic cost of carrying stores (C_{Stores}, kJ·month^{-1}) is a function of E_{Stores} (calculated within the parentheses on the right side of eq. 9) and is calculated as

$$(9) \qquad C_{\text{Stores}}(E_{\text{Stores}}) = \tau \cdot (W_{\text{Total}}^{\lambda} - W_{\text{Structure}}^{\lambda}) \cdot H_{\text{Adj}}(K)$$

The value for τ implies that the mass-specific cost of maintenance of energy stores is less than the cost of maintaining other tissue because lipids are less metabolically active than somatic tissue. The exponent λ follows the allometric scaling of metabolic rate (Fitzgibbon et al. 2008), and H_{Adj} (unitless, ranging between 0 and 1) is the adjustment of metabolic costs resulting from the influence of energy stores, producing a more bulky appearance with less efficient hydrodynamics (Vogel 1994). The value for this adjustment is calculated as

$$(10) \qquad H_{\text{Adj}}(K) = \left(\frac{K}{K_{\text{Min}}}\right)^{v}$$

where v (unitless) scales the increase in H_{Adj} with increasing values for K.

Energy allocation

Available energy is allocated between growth and energy storage at each time step (Roff 1983). The proportion of energy directed toward energy storage is u, which is a variable assigned at each time step in the model based on results from the optimizing algorithm portion of the model. The values of u and I determine the change in values for E_{Stores} and $W_{\text{Structure}}$ (and, consequently, L).

If I_{Net} is positive, then the energy that is available for tissue production is calculated as

$$(11) \qquad E_{\text{Prod}} = I_{\text{Net}} \cdot C_{\text{s}}$$

where C_{s} is the proportion of assimilated energy required to synthesize new tissue (Wieser 1994). Energy available to production is then allocated to energy stores as

$$(12) \qquad E_{\text{Stores}}(t+1|u) = E_{\text{Stores}}(t) + u \cdot E_{\text{Prod}}$$

and the change in $E_{\text{Structure}}$ as

$$(13) \qquad E_{\text{Structure}}(t+1|u) = E_{\text{Structure}}(t) + (1-u) \cdot E_{\text{Prod}}$$

If I_{Net} is negative, then energy is drawn from energy stores to meet the net energy deficit as

$$(14) \qquad E_{\text{Stores}}(t+1) = E_{\text{Stores}}(t) + I_{\text{Net}}$$

The value for $W_{\text{Structure}}(t+1)$ is calculated as

$$(15) \qquad W_{\text{Structure}}(t+1) = \frac{E_{\text{Structure}}}{\rho_{\text{Structure}}}$$

where $\rho_{\text{Structure}}$ (kJ·g^{-1}) is the energy density of somatic tissue. The value for $W_{\text{Stores}}(t+1)$ is calculated based on eq. 2. A new value for $W_{\text{Total}}(t+1)$ is calculated from the values for $W_{\text{Structure}}(t+1)$ and $W_{\text{Stores}}(t+1)$ as in eq. 3. Based on the calculation for Fulton's condition factor $K = 100W \cdot L^{-3}$, the new length of the fish is calculated as

$$(16) \qquad L(t+1) = \left[\frac{100 \cdot W_{\text{Structure}}(t+1)}{K_{\text{Min}}}\right]^{\frac{1}{3}}$$

Migration and spawning

Tagging studies find that large, presumably mature, ABFT have complex and varied movement patterns as they move between the feeding and potential spawning grounds (Block et al. 2005; Sibert et al. 2006; Galuardi et al.2010). Furthermore, little is known regarding the amount of feeding that occurs during migration and spawning, though based on depth patterns from electronic tags and stable isotope analysis (Estrada et al. 2005; Logan 2010), it is likely that ABFT feed during both. Regardless, movement between feeding and spawning areas requires time and energy expenditure. To simplify, time and energetic cost of migration and spawning is compartmentalized in discrete periods in the model. Note that time involves an opportunity cost, as the tuna cannot perform other activities while migrating or spawning, while the energetic cost has implications for energy stores required. Because of this, the model includes both time and energetic costs, and these are part of different trade-offs that are linked with size (size affects the energy costs of swimming and the timing of migration through swimming speed). The feeding and spawning areas themselves are not spatially explicit, yet the two areas are separated by a specified distance. We assumed that tuna spend time either in a feeding area or a spawning area or migrate between these two areas. For migration, ABFT make directed movements between the feeding and spawning areas based on an annual cycle driven by a spatially and temporally fixed spawning schedule, but may forage underway. Fish utilize a single spawning area, and spawning includes all behaviors and activity associated with spawning. Spawning occurs in May both in the Gulf of Mexico and the Mediterranean (Mather et al. 1995). The model allows for sufficient time for the fish to arrive on the spawning grounds by 1 May. Following spawning at the end of May, the fish return to the feeding area. The following calculations are used to determine the energetic cost of spawning and the amount of energy that is put towards egg production.

Migration time

The migration time (T_{Mig}, months) is a function of the distance between the feeding and spawning areas (D_{Mig}, m) and the speed that the fish travels during migration ($U_{\text{Opt}}(L)$, m·month^{-1}) and is calculated as

$$(17) \qquad T_{\text{Mig}} = \frac{D_{\text{Mig}}}{U_{\text{Opt}}(L)}$$

The fish is assumed to travel at an optimal cruising speed according to

$$U_{Opt} = r \cdot L^{e} \quad (18)$$

where parameters r and e are based on a relationship for pelagic fish (Ware 1978). As an example, migration speed and time are shown as a function of fish length (Fig. 2).

Migration costs

We assumed that the metabolic costs during migration scale according to the time required for migration and the physical power required for fish movement through the water column. The metabolic cost of migration to and from the spawning area is calculated as

$$C_{Mig} = 2 \cdot T_{Mig} \cdot \left(\alpha \cdot L^{\beta} \cdot U_{Opt}^{\varepsilon}\right) \quad (19)$$

where β and ε (unitless) are scaling exponents drawn from a study of the relationship between fish length and power required for movement at an optimal cruising speed (Ware 1978). The coefficient α is tuned so that the metabolic cost is equivalent to twice the routine metabolic rate (see spawning metabolic rate calculation below) for a 200 cm fish. (Ware (1978) found that fish metabolic rate while swimming at an optimal speed was two times routine metabolic rate.) The factor 2 is to account for migration both ways.

Spawning costs

Metabolic costs for ABFT during spawning are unknown (Mather et al. 1995). Therefore, metabolic rate during spawning is estimated according to Fitzgibbon et al. (2008), who measured routine metabolic rate (RMR, kJ·month^{-1}) for non-spawning southern bluefin tuna (*Thunnus maccoyii*) in holding pens while traveling approximately 100 cm·s^{-1}:

$$\mathrm{RMR} = \varpi \cdot W_{Total}^{\varphi} \quad (20)$$

This metabolic rate during spawning was adjusted for temperature as follows:

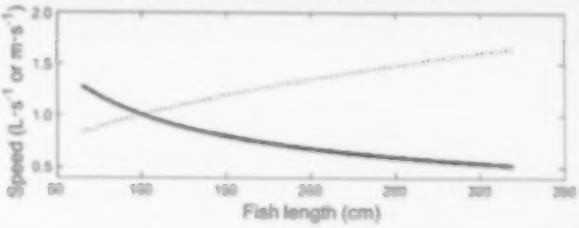
$$\mathrm{RMR}_{TAdj} = \mathrm{RMR} \cdot Q_{10}^{\frac{T_w - 25}{10}} \quad (21)$$

where T_w is water temperature (°C). The value for Q_{10} is representative of the metabolic influence of temperature for fish (White et al. 2006) and was used by Fitzgibbon et al. (2008) for tuna species. The temperature-adjusted metabolic rate was then adjusted for activity (assumed to be 25% higher than temperature adjusted routine metabolic cost) during spawning, giving the metabolic cost of spawning (C_{Spawn}, kJ·month^{-1}):

$$C_{Spawn} = \mathrm{RMR}_{TAdj} \cdot \mathrm{Act} \quad (22)$$

Temperature is usually much higher on the spawning grounds than on the feeding grounds, particularly for W-BFT when in the Caribbean. Although temperature has a profound influence on physiological rates, tuna are known to make extensive vertical migrations to different temperature regimes during the spawning migration and to a lesser degree during spawning itself (Block et al. 2001). As their large body sizes may buffer temperature fluctuations in the shorter term, the overall effect of warmer surface waters on energetic costs is uncertain. Because the model does not include vertical migration behavior explicitly, we therefore choose T_w = 24 °C, which is a typical temperature experienced by ABFT during spawning in the Gulf of Mexico (Mather et al. 1995; Garcia et al. 2005; Teo et al. 2007*a*).

Fig. 2. Migration time speed as it varies with fish length (L). Speed is shown in L·s^{-1} (solid line) and m·s^{-1} (dotted line).



Intake while migrating and spawning

Although ABFT appear to feed sporadically during migration and spawning (Mather et al. 1995), we assumed that fish do not add to their energy stores during this period, but that feeding does occur and compensates for some proportion of metabolic costs during these activities. The total metabolic cost of migration and spawning ($C_{MigSpawn}$, kJ·month^{-1}) is then calculated as

$$C_{MigSpawn} = (C_{Mig} \cdot F_{Mig}) + (C_{Spawn} \cdot F_{Spawn}) \quad (23)$$

where F_{Mig} (unitless) and F_{Spawn} (unitless) are the proportions of metabolic costs not met by feeding during migration and spawning (including activity but excluding gonads), respectively.

Energy to egg production

A spawning bluefin tuna preserves some of its stored energy to maintain survival. Therefore, spawning fish only use stored energy that will allow for the metabolic costs of migration and spawning and that will leave a fish in a particular condition upon return to the feeding ground ($K_{PostSpawn}$, unitless). The remaining stored energy is spawned, and the energy put toward egg production (E_{Egg}, kJ) is calculated as

$$E_{Egg} = \left[E_{StoresPreSpawn} - C_{MigSpawn} - \frac{(K_{PostSpawn} - K_{Min}) \cdot L^3 \cdot \rho_{Stores}}{100}\right] \cdot C_s \quad (24)$$

where $E_{StoresPreSpawn}$ (kJ) is the stored energy prior to migration and spawning. The efficiency of biosynthesis C_s is used again and represents the energetic cost of transforming stored fat and protein to eggs in the gonads. The factor 100 in the denominator is a conversion for Fulton condition when length is expressed in centimetres and mass in grams. We do not include larval growth and survival explicitly. Recruits are introduced at age 18 months, which implicitly assumes that mean survival from spawned egg to age 18 months does not vary systematically with the mother's phenotype or the environment in the model.

Mortality

A background mortality rate (M_c, year^{-1}) is imposed on fish evenly throughout the year regardless of its activity, so that survival probability (S_c, month^{-1}) is calculated as

$$S_c = \exp\left(\frac{-M_c}{12}\right) \quad (25)$$

In addition, fish that have a condition below a critical Fulton's K (K_{Min}) value die, such that $S_c = 0$.

We recognize that fish experience both natural and fishing mortalities, and mortality may vary with size, age, or whether a fish is spawning, migrating, or in a feeding area. Furthermore, modeling has suggested that where and when mortality occurs is important for influencing emerging population characteristics such as growth, age-at-maturity, and reproductive effort (Law and Grey 1989; Dunlop et al. 2009). However, a simple, integrated mortality term was used in this study because of a lack of extensive data on variability in natural and fishing mortality. An additive approach to this term would have introduced further unknown parameters and was not required based on the objectives of this study.

Optimizing algorithm

Dynamic programming is used to optimize life history strategies (Houston and McNamara 1999; Clark and Mangel 2000), which produces a "decision map" that guides energy allocation and the decision to spawn based on individual state values in the forward simulations (see next section: "Forward simulations: initialization and exploratory scenarios"). This approach begins at a maximum age (when all fish die from senescence and future reproductive success is assumed to be zero) and, working backwards in time, optimizes the allocation of energy to somatic growth versus energy stores (for reproduction or survival during low prey availability). As a fitness measure, the model maximizes expected lifetime egg production. The maximum age used in this study is 25 years, and the maximum size was set at 350 cm. Although older ABFT have been observed (Neilson and Campana 2008), a 25-year maximum age was used because of the scarcity of fish older than this age (Mather et al. 1995). The optimization problem then finds the energy allocation (u) for each combination of variables that are available to the individual (state variables: age a, body length L, stored energy E, food environment ϕ) that maximizes expected future reproductive output (V) at each decision point:

$$V(a, L, E, \phi) = \max_u \left\{ b(E) + S \sum_{\phi(t+1)} P[\phi(t+1)|\phi(t)] \times V[a+1, L(t+1|u), E(t+1|u), \phi(t+1)] \right\} \quad (26)$$

Here, $b(E)$ is the fecundity when spawned, S is survivorship, and $P[\phi(t+1)|\phi(t)]$ is the probability of realizing a food environment in the next time step, given the value of the food environment in the current time step. $L(t+1|u)$ and $E(t+1|u)$ are the new states in the next time step, depending on the energy allocation u. If the individual survives, its future expected reproductive value is then given by $V[a+1, L(t+1|u), E(t+1|u), \phi(t+1)]$.

Two additional optimizations are made during the backwards iteration. First, the postspawning condition ($K_{\text{PostSpawn}}$) that produces the maximum expected future egg production is calculated during the spawning period for each combination of state variables. The optimal postspawning condition depends on a balance between benefits gained from investment in a current reproductive effort and those gained by increased future survival and reproduction (as a result of preserving greater energy reserves when returning to the feeding area the following season). Second, during the spawning period, the fitness value for both spawning and nonspawning are calculated and the behavior that maximizes fitness is recorded for each combination of state variables. When fish do not spawn, they produce no eggs, but are able to allocate energy to growth and do not incur the energetic costs of migration and spawning.

Overall, the optimizing algorithm produces a life history plan that guides (*i*) allocation of energy, (*ii*) the decision to spawn, and (*iii*) postspawning condition as a function of each combination of state variables during the forward simulation. The critical assumption here is that individuals, either through selection or through phenotypic plasticity, behave optimally regarding these three decision sets as they interact with a particular environmental scenario.

Forward simulations: initialization and exploratory scenarios

Forward simulations of the model begin for age class 1 (year) fish in January (approximately 18 months) at a size of 65 cm (see Mather et al. 1995) and a Fulton's K of 1.5. The forward simulation was run for a 2000-year period under each environmental scenario. Such a long simulation is needed to record the mean and variability of the response in that environment, as averaging over time is the same as averaging over environments when the environment is fluctuating around an equilibrium. Fish were allowed to skip spawning in any given year, and age-at-maturity was defined as the age that fish first spawn. Population characteristics were recorded during the second 1000 years of simulations, thus allowing for the model to stabilize during the first 1000 years. A parameter space for a reference scenario (baseline values in Tables 1–3) was selected to facilitate validation of the model through comparison of model output with observations of growth (Turner and Restrepo 1994; Neilson and Campana 2008; Restrepo et al. 2010), age-at-maturity (Turner et al. 1991; Goldstein et al. 2007), and seasonal changes in condition (W. Golet and M. Lutcavage, unpublished data) for W-BFT. We then use the model to test whether the population characteristics can be altered to match those of E-BFT by increasing mortality and decreasing migratory costs. In place of a potentially overwhelming set of sensitivity studies involving all parameters, a subset of primary sensitivity simulations were carried out with parameters that are central to the model and may vary among ABFT subpopulations (Table 2). These simulations were designed to give insight into the sensitivity of the model to variability among uncertain parameters while providing some insight into factors that may be responsible for observed variability in life history patterns among ABFT subpopulations. These simulation sets investigated the influence of variability among mortality rate and spawning migration distance, expressed as the proportion of energetic costs

Table 1. Parameters used in the Atlantic bluefin tuna life history model.

Symbol	Definition	Value and unit	Source
K_{Min}	Minimum value for Fulton's K	1.4 (unitless)	W. Golet and M. Lutcavage, unpublished data
K_{Max}	Maximum value for Fulton's K	2.1 (unitless)	W. Golet and M. Lutcavage, unpublished data
ρ_{Stores}	Wet mass energy density of stored lipids	8.7×10^6 kJ·kg^{-1}	Assumed similar to *Gadus morhua*; Jørgensen and Fiksen 2006
$\rho_{Structure}$	Wet mass energy density of somatic tissue	4.2×10^6 kJ·kg^{-1}	Assumed similar to *Gadus morhua*; Holdway and Beamish 1984
b	Scaling of the intake calculation	2.4 (unitless)	Overholtz 2006; Fitzgibbon et al. 2008
S_{Mean}	Mean value of seasonality over a year	0.65 (unitless)	Set by fitting seasonal growth to observations; see Fig. 3*b*
$\bar{X}$	Mean value for stochasticity (X)	0.0 (unitless)	Tuned to give interindividual variability that fits reasonably well with observations
C_2	Variance in stochasticity (X)	0.4 (unitless)	
C_3	Scaling of stochasticity (X)	0.4 (unitless)	
λ	Scaling of metabolic cost of energy stores	0.86 (unitless)	Fitzgibbon et al. 2008
S_y	Cost of biosynthesis	0.77 (unitless)	Wieser 1994
r	Coefficient for calculation of optimal cruising swim speed	0.138 s^{-1}	Ware 1978
e	Scaling factor for calculation of optimal cruising swim speed	0.43 (unitless)	Ware 1978
β	Scaling with length of cost of migration	1.42 unitless	Ware 1978
ε	Scaling with swim speed of cost of migration	2.42 unitless	Ware 1978
m	Coefficient for RMR calculation	4.08×10^6 kJ·month^{-1}	Fitzgibbon et al. 2008
ψ	Scaling of RMR with total mass	0.89 (unitless)	Fitzgibbon et al. 2008
Q_{10}	Scaling of metabolic rate with temperature (at temperature of 25 °C)	1.67 (unitless)	White et al. 2006
T_w	Water temperature experienced during spawning	24 °C	Mather et al. 1995; Garcia et al. 2005; Teo et al. 2007*a*
—	Spawning month	May	Mather et al. 1995

Note: RMR, routine metabolic rate.

Table 2. Parameters varied in primary sensitivity simulations of the Atlantic bluefin tuna life history model.

Parameter	Definition	Baseline: sensitivity range	Rationale for values
M	Annual mortality rate	0.34: 0.10 to 0.45 (year^{-1})	Natural mortality (ICCAT 2007) plus variable fishing mortality
D_{Mig}	Migration distance	3500: 500 to 4500 (km)	Mather et al. 1995; Block et al. 2001, 2005; Galuardi et al. 2010
F_{Mig}	Proportion of metabolic cost during migration that is compensated for by feeding		
F_{Spawn}	Proportion of metabolic cost during spawning (excluding energy to gonads) that is compensated for by feeding	0.025: 0.15 to 0.01 (unitless)	Tuned to allow survival and to match observed growth and maturity schedules

Note: Exploratory range and the baseline value are given. Sources are indicated where applicable; otherwise, rationale or method for choice is given. Results provided in Figs. 8 and 9.

covered by feeding during migration and spawning (Table 2). We selected the range of integrated mortality rate to encompass an estimated natural mortality (0.10 to 0.24 year^{-1}; ICCAT 2007, 2009) plus reasonable fishing mortality (bringing total morality to 0.45 year^{-1}). The range of migratory distances used in these simulations encompass shorter spawning migrations that have been observed for E-BFT (500–1000 km; Mather et al. 1995; Block et al. 2005) and longer migrations recorded for W-BFT (3500 km; Block et al. 2001, 2005; Galuardi et al. 2010). The influence of variability in the proportion of metabolic costs incurred during migration and spawning on population characteristics were conducted over a range of plausible values. Secondary sensitivity simulations were carried out for additional poorly constrained parameters (Table 3), again over a range of plausible values.

Results

Model validation and reference simulation

The model's reference simulation produced indeterminate growth, with the fastest growth rate during the fish's first 8 years (Fig. 3*a*). Growth rate is then reduced before reaching an asymptote at around 275 cm when fish are >21 years. For fish >15 years, the simulated fish are smaller than is predicted by the W-BFT growth curve and slightly larger than is predicted according to the E-BFT growth curve. However, the size-at-age curve for W-BFT is uncertain, and the modeled size-at-age values for older fish are slightly larger than data from a recent study that aged W-BFT (Neilson and Campana 2008; Restrepo et al. 2010). Juvenile growth follows a seasonal pattern similar to that observed in W-BFT

Table 3. Parameters varied in secondary sensitivity simulations of the Atlantic bluefin tuna life history model.

Parameter	Definition	Sensitivity range (baseline: range)	Rationale for baseline values	Effect of parameter increase on growth	Effect of parameter increase on maturity schedule
Act	Spawning activity adjustment	1.25: 0.625–1.875 (unitless)	To allow for increased behavioral activity during mating and spawning	Slight decrease	Slight delay in maturity
a	Intake coefficient	1.8: 1.62–1.98 (unitless)	Set to match observed growth and condition changes	Increase	Slight delay in maturity
τ	Coefficient for metabolic cost of energy stores	3.16×10^{4}: $1.58–4.75\times10^{4}$ ($kJ\cdot kg^{-1}\cdot month^{-1}$)	Set lower than for somatic tissue, as stored lipids generally are less metabolically active	Increase	Delayed maturity
v	Scaling of the hydrodynamic cost of energy stores	2.0: 1.0–3.0 (unitless)	Drag scales as cross-sectional area (Vogel 1994); this exponent assumes further efficiency loss at higher condition factors	Increase to 14 years, then decrease	Slightly earlier maturity
S_{Amp}	Seasonality amplitude (maximum value)	0.35: 0.175–0.525 (unitless)	Set by fitting seasonal growth to observations; see Fig. 3*b*	Increase	Delayed maturity
D_{Peak}	Seasonality peak (timing)	15 Aug.: 1 Aug. – 1 Sept.	Maximum seasonal mass gain; W. Golet and M. Lutcavage, unpublished data	(Later peak) decrease to 12 years, then increase	(Later peak) earlier maturity
C_1	Autocorrelation	0.90: 0.30–0.99 (unitless)	Tuned to give interindividual variability that fits reasonably well with observations	Slight increase	Slightly earlier maturity
α	Coefficient for cost of migration	6.51×10^{4}: $1.30–1.17\times10^{4}$ ($kJ\cdot m^{-2}$)	Tuned to be twice RMR for a 200 cm fish; see also Ware 1978	Little change	Delayed maturity

Note: These parameters were chosen because of uncertainty over their values. Sources are indicated where applicable, otherwise rationale or method for choice of baseline parameter value is given. Results are summarized below and presented in Appendix A, Fig. A1.

(Fig. 3*b*). The observed mean monthly W-BFT Fulton's K between May and November for mature fish compares favorably with increases in condition over the same time period produced by the model (Fig. 3*c*). Older, mature fish maintained a higher Fulton's K during the year, largely because a higher U_{Opt} allowed them to arrive on the feeding grounds earlier in the year to recover stores lost during migration and spawning. Juvenile fish allocated energy primarily to growth and allocated to energy stores only to maintain a stable Fulton's K value of around 1.54 throughout the year. Under reference conditions, fish matured between the ages of 6 and 11 years (150–235 cm), with age at 50% maturity at 8 years (210 cm) (Fig. 3*c*). The size- and age-at-maturity under the reference conditions were within the range of estimations based on field data and were more similar to estimates of the maturity schedule for W-BFT (7–10 years; Turner et al. 1991; Goldstein et al. 2007) than for E-BFT (3–5 years; Tiews 1963; Medina et al. 2002; Corriero et al. 2005).

To demonstrate the evolution of an individual life trajectory under reference conditions, fish length, food environment, condition, fish mass, energy in egg production, and allocation of energy during a 25-year lifespan are shown (Fig. 4). This particular fish began spawning in its ninth year when it was 200 cm and demonstrated a seasonal and maturation-stage pattern in energy allocation (Fig. 4*f*). During the juvenile period, this fish allocated energy primarily toward growth, with seasonal increase in energy directed to storage in winter when net energy intake is low. After maturation, the adult fish primarily favored energy storage, with a brief period of partial energy allocation to growth immediately following return from the spawning grounds.

Skipped spawning

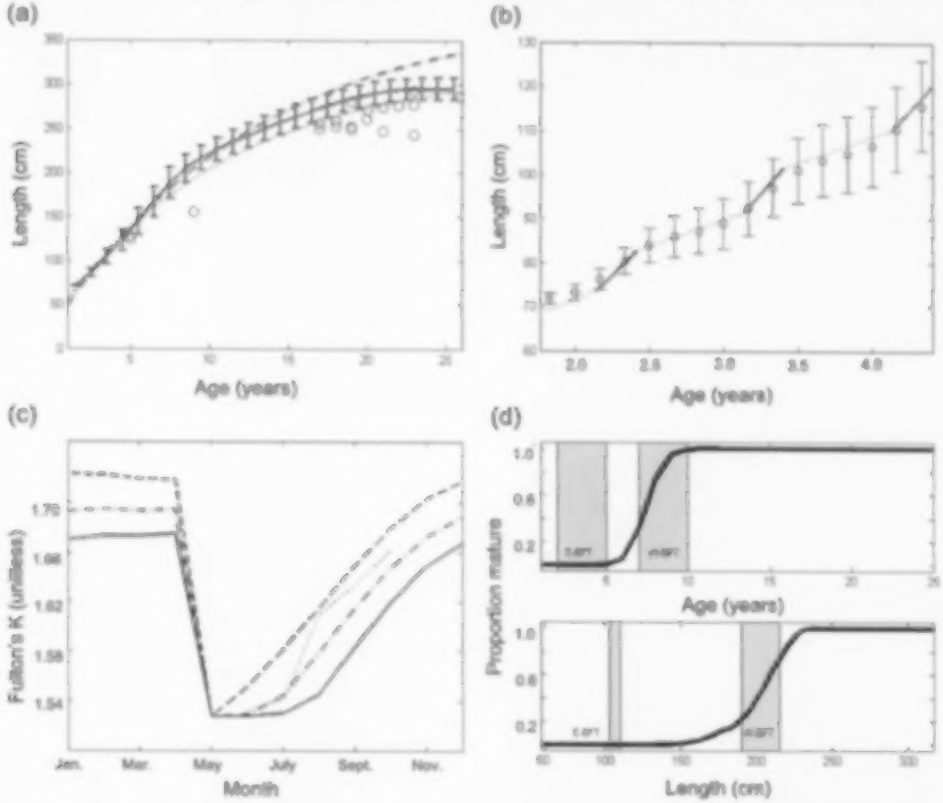
Under reference conditions, skipped spawning occurred primarily among younger, recently matured fish. The proportion of individuals that skipped spawning decreased quickly with age from age 7 (the first opportunity for skipped spawning) to 13 years, and no fish older than 13 years skipped spawning (Fig. 5*a*). Under the reference conditions, 42% of fish spawned during their eighth year. In comparison with these, skipped spawners were in lower condition, weighed less, and were shorter just prior to migration (Figs. 5*b*–5*d*).

The mean growth curve was strongly influenced by the age-at-maturity schedule. In general, mean growth curves exhibit a reduced rate of growth following maturity as those fish begin to allocate energy to both growth and reproduction rather than solely to growth. This results in a reduced size at a particular age for an earlier maturing fish population, but only after some proportion of that population matures (Fig. 6).

Eastern bluefin tuna

To shift the reference simulation that resembled population characteristics of W-BFT to those of E-BFT, it was necessary to (*i*) reduce the reproductive costs by reducing the migration

Fig. 3. (*a*) Comparison of length-at-age from model output (mean and standard error) for the reference scenario (solid line) with growth data from the western Atlantic from Neilson and Campana (2008) (open circles) and the ICCAT growth curves for W-BFT (dot-dashed line) and E-BFT (dotted line). (*b*) Juvenile growth predicted by the model (open circles, with standard error) and based on data from the western North Atlantic (Mather and Shuck 1960; solid line = data; dotted line = interpolation). (*c*) Model output for the reference condition for monthly mean Fulton's *K* condition index for 10- (solid line), 18- (dot-dashed line), and 24- (dashed line) year-old fish and mean monthly value of Fulton's *K* for fish between 8- and 20+-year-old fish in the Gulf of Maine (dotted line) between June and November from catch data between 1984 and 2007. (*d*) The proportion of spawning individuals as a function of age (top) and size (bottom) produced through 1000 simulations of the model under reference conditions. The current estimates of age- and size-at-maturity for E-BFT and W-BFT are indicated.



distance (from 3500 to 500 km) and increase the proportion metabolic costs met by feeding during spawning and migration (from 0.985 to 0.995) and (*ii*) increase the mortality rate to 0.37. This produced a growth curve (Fig. 7*a*) and age-at-maturity (Fig. 7*b*) similar to what is currently accepted for E-BFT. Length at 50% maturity was similar to that which is accepted for E-BFT (Fig. 7*b*), though maturity occurs over a broad range of lengths (70–170 cm) and does not increase linearly over that range. Instead, local peaks in proportion spawning occur over this length range. This pattern reflects that only the largest fish within the youngest age classes are spawning. The compounding effects of environmental stochasticity smooth these peaks as fish age and age classes increasingly overlap in length. As was the case for the W-BFT conditions, skipped spawning occurs only for the youngest recently matured fish (ages 3 to 5 years; Fig. 7*c*).

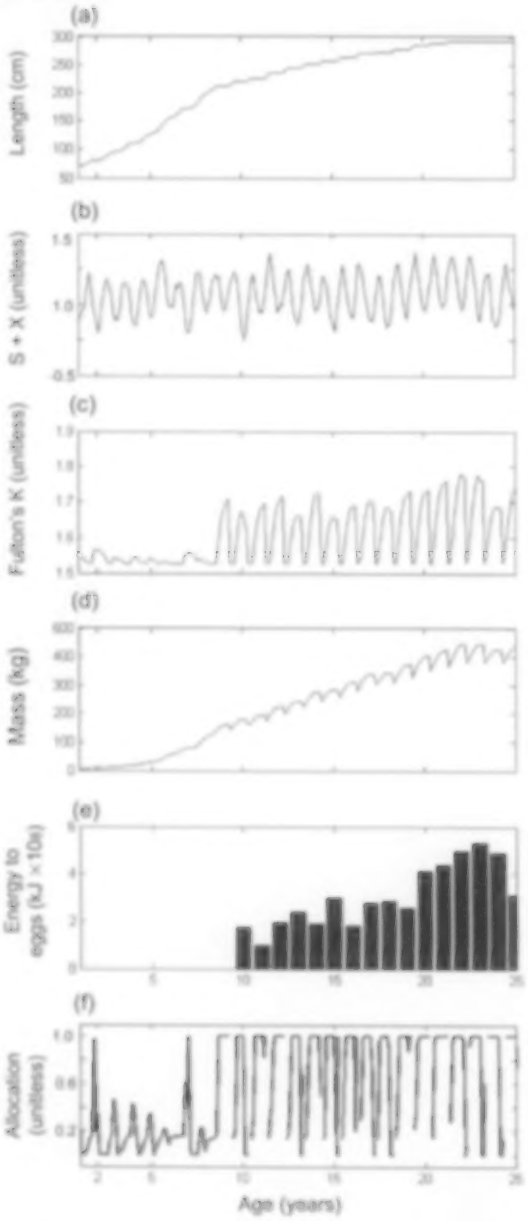
Sensitivity analyses

The age at 50% maturity ranged between 13 and 3 years as annual mortality rate was varied between 0.1 and 0.45 (year^{-1}), and the spawning migration distance was varied between 500 and 4500 km (Fig. 8). Over this range of variability, the age at 50% maturity decreases as annual mortality rate increases and increases with increasing spawning migration distance. The same range in mortality rate, combined with the proportion of metabolic costs met by feeding during migration and spawning (ranging between 0.975 and 1.000), produced an age at 50% maturity between 4 and 14 years (Fig. 9). (Results for the secondary sensitivity simulations are summarized in Table 3 and are included in Appendix A.)

Discussion

The reference simulation recreates observations of growth, age-at-maturity, and seasonal variability in condition for W-BFT, although we acknowledge that their maturity and reproduction schedules are not well known. This suggests that the model structure and parameter settings provide information necessary to adequately represent the interaction between the environment and ABFT life history. This gives us confidence in the model structure and parameterizations and allows us to

Fig. 4. An individual example of fish length (*a*), food environment (*b*), condition (*c*), mass (*d*), energy put toward eggs (*e*), and the proportion of energy allocation to energy stores (*f*; no values are plotted when there is no ingested energy to allocate, e.g., during spawning) over the 25-year life of a BFT under reference conditions in the model.



explore and discuss some of the model components and parameters that are important for model behavior.

Net energy intake and seasonality

While integration of ingested energy and metabolic costs into net energy intake limited the ability of the model to resolve differences in specific sources of environmental variability (e.g., prey quality, cost of foraging), we avoided major difficulties by using this approach. ABFT have extremely high energy demands, requiring equally high energy intake rates. Therefore, energy available for allocation to growth and energy stores is the difference between two values that are extremely difficult to estimate, and using an additive approach to calculate net energy intake made it difficult to achieve stable model dynamics. The simplifying assumption of an integrated net energy intake function allowed for more stable model behavior and allowed us to easily add seasonality to the model. Given the uncertainty and complexity in food quality, abundance, and metabolic rate over a bluefin's lifespan, this was highly desirable.

The seasonality function is a central component of the model that is responsible for accurately predicting juvenile growth and the increase in fat stores for larger adults during the summer and autumn feeding period. It is also a critical process in determining age-at-maturity because the benefit of migration and spawning is weighed against the benefits associated with feeding opportunities missed while migrating and spawning, which are inherent to the seasonality function. Spawning (May) occurs just after the net energy intake is at its lowest value (March), and the return migration in June and July occur as the seasonality function is increasing toward its August peak. Larger fish, because they are faster swimmers, are able to return to productive waters earlier and thus are able to capitalize on the increasingly favorable food conditions there. This represents an incentive for allocation to growth (along with increased ability to ingest and store energy), and the strength of this incentive varies with the phenology of peak seasonal energy intake. When this seasonality peaks earlier than 15 August, the incentive for growth is greater and age-at-maturity is delayed because larger fish are able to return from spawning to capitalize on earlier peak in seasonality. When seasonality peaks later, the incentive for growth is reduced, and age-at-maturity is younger because extended migration time for smaller fish does not influence their access to peak seasonality in energy intake. This highlights the importance of phenology and the complex costs and benefits linked to the timing of migration with respect to missed feeding opportunities.

Seasonality and environmental stochasticity were responsible for the postspawning condition having minimal or low variability in the simulations. This result reflects a poor correlation between the food environment immediately prior to and following migration and spawning, leaving ABFT unable to anticipate the food environment following spawning. This could also indicate that ABFT experience a relatively high and increasing net energy intake when they return to the feeding grounds and thus would not draw on their energy reserves. Therefore, we expect that higher optimal postspawning condition could result if fish experience lower forage quality upon their return to the feeding area (risking starvation) or if there is lower autocorrelation in the food environment (preventing fish from anticipating the food environment when they return to the feeding area based on conditions when they left for spawning).

Feeding during migration and spawning

The model is sensitive to the proportion of metabolic costs

Fig. 5. The rate that mature fish skip spawning (*a*) and the mean and standard deviation of Fulton's *K* (*b*), fish weight (*c*), and length (*d*) in March (month when spawning decision is made) for fish that spawn and those that do not spawn in their eighth year.

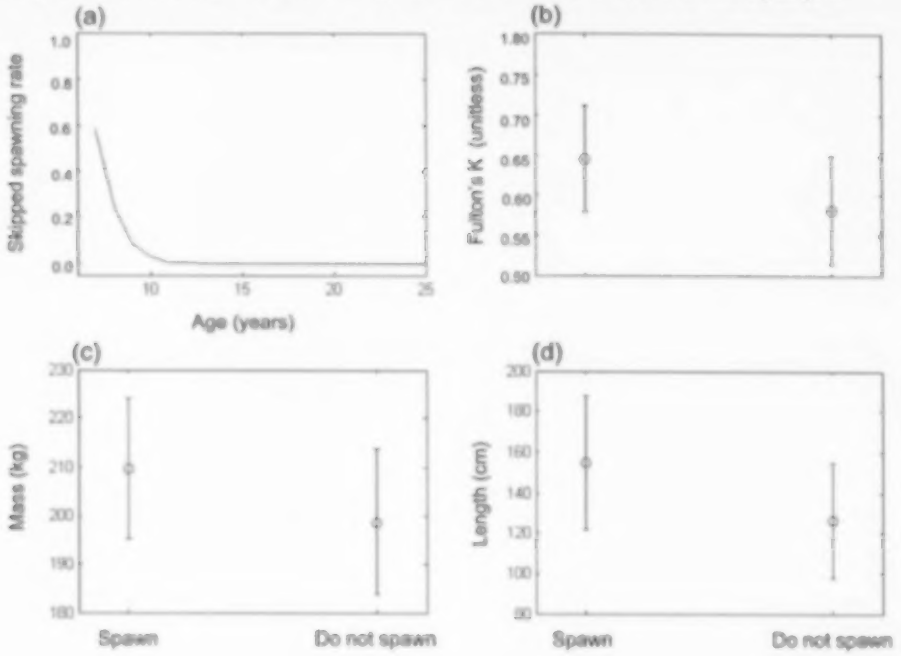
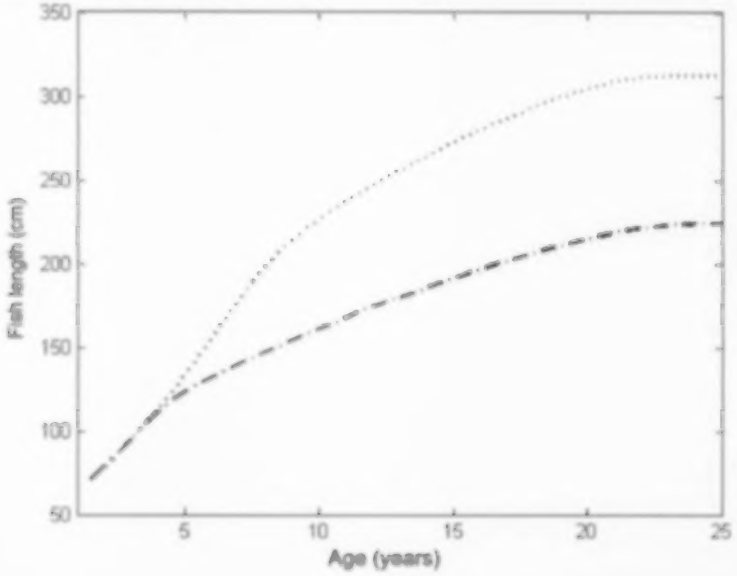


Fig. 6. Difference in growth for fish with a migration distance of 2500 km and age at 50% maturity of 4 years (dot-dashed line, mortality = 0.45) and 8 years (dotted line, mortality = 0.33). The remaining parameters are set at reference values.



during migration and spawning that are covered by feeding. Large-scale migration presumably links individuals with habitat that is beneficial for an incipient developmental stage (e.g., migration between spawning and nursery habitats) or for larval development and survival (spawning migrations). Complex trade-offs should structure ABFT migration that balances benefits to larvae (e.g., food availability, lack of predators, favorable temperatures for growth) or adult condition with costs incurred by migrating and spawning (e.g., lack of food, thermal stress).

ABFT larval habitat requirements are not completely understood, though evidence suggests that most larvae re-

Fig. 7. (*a*) Comparison of length-at-age from model output (mean and standard error) for the scenario for E-BFT (solid line) with the ICCAT growth curves for W-BFT (dotted line) and E-BFT (dot-dashed line). (*b*) The proportion of spawning individuals as a function of age (top) and size (bottom) produced through 1000 simulations of the model under a possible E-BFT scenario. The current estimates of age- and size-at-maturity for E-BFT and W-BFT are indicated. (*c*) The rate that mature fish skip spawning for the E-BFT scenario.

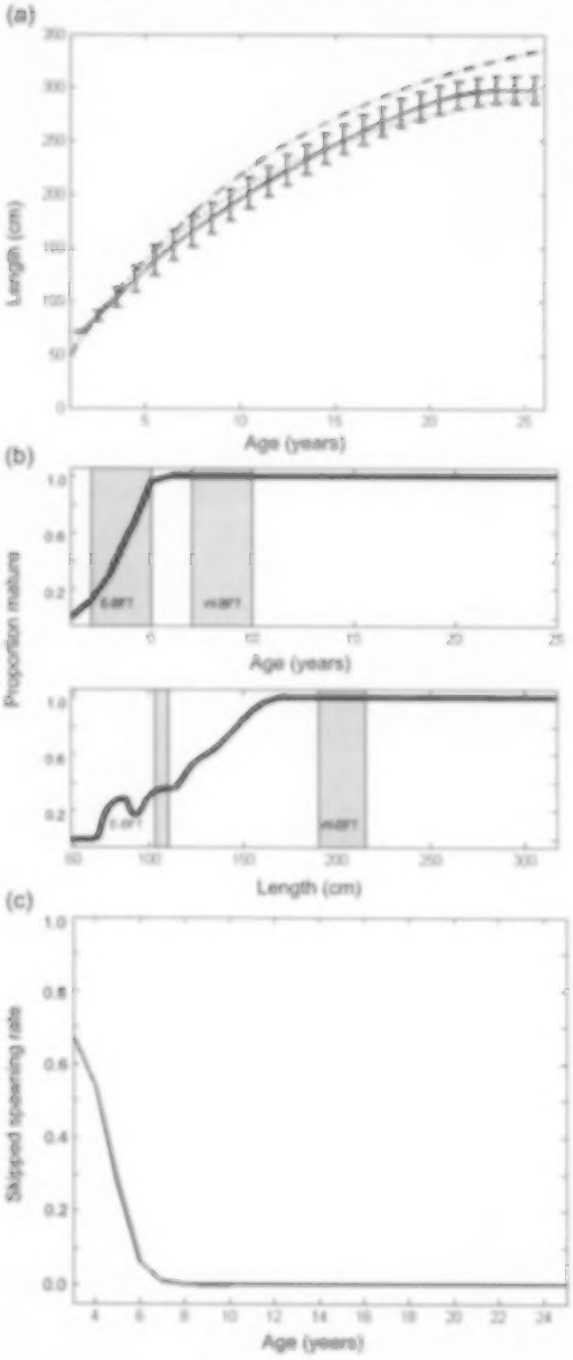


Fig. 8. The influence of variability in the spawning migration distance and cumulative annual mortality rate on age-at-maturity (50%).

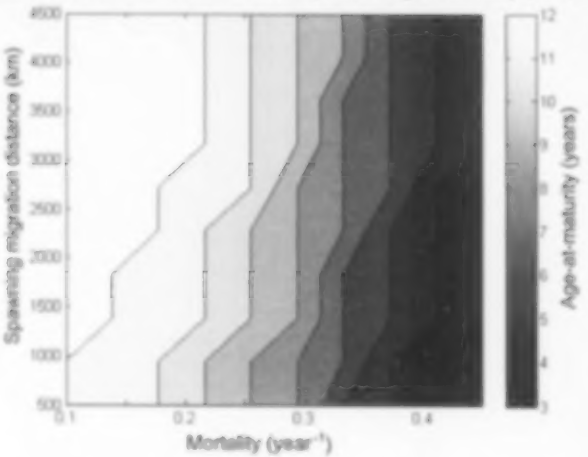
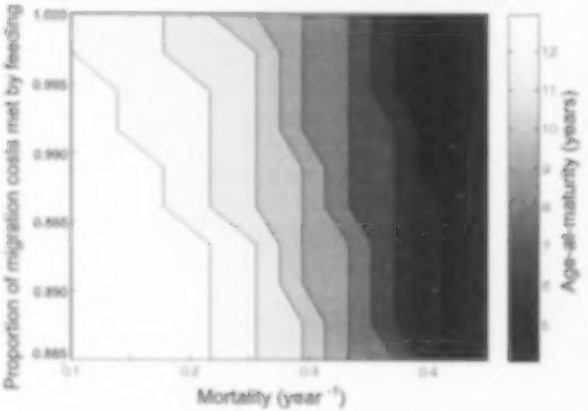


Fig. 9. The influence of variability in the proportion of metabolic costs met through feeding during a 3500 km migration and spawning and cumulative annual mortality rate on age-at-maturity (50%).



quire temperatures of at least 24 °C (Mather et al. 1995; Garcia et al. 2005). Although appropriate temperatures are necessary for larval development, the distribution and quality of food and predator density are also crucial factors. Improving our understanding of the complex interactions among factors influencing early life history that combine to determine the spatial and temporal variability of suitable spawning habitat (e.g., Lehodey et al. 2008) must be accomplished in future modeling efforts.

From the perspective of the adult, spawning areas are up to 20 °C warmer than their primary feeding areas (possibly introducing thermoregulatory stress), and their suitability as forage grounds for adult ABFT is not known. Uncertainty regarding adult migration and spawning energy requirements, thermal stresses, and feeding activity further limits our understanding of factors that structure trade-offs responsible for ABFT spawning habitat distributions and migration behaviors, though some studies have been able to provide relevant information on this topic.

For example, Pacific bluefin tuna (*Thunnus orientalis*) mi-

grate 8000 km from the western to the eastern Pacific during their first and second year along the Subarctic Frontal Zone (Inagake et al. 2001; Kitagawa et al. 2009), a behavior that may provide a beneficial thermal environment with high prey availability during migration (Kitagawa et al. 2009). Similar strategies for migrating ABFT are not well studied, although juveniles from 1–2 years make trans-Atlantic crossings (Mather et al. 1995) For W-BFT that migrate to the Gulf of Mexico during the putative breeding period, Teo et al. (2007*b*) found a directed movement pattern during migration away from summer feeding areas, suggesting a strategy that minimizes travel time and allows for only opportunistic feeding along the way. However, Galuardi et al. (2010) demonstrate complex and varied movement patterns (e.g., varied departure time from feeding areas, significant nondirected movement during migration, and varied residency periods within and outside of the Gulf of Mexico during the putative spawning period) of adult ABFT in the western Atlantic, suggesting more substantial feeding during the migration and spawning periods and possibly alternative spawning areas or frequent skipped spawning. Overall, little is known about migration strategies and the change in condition of ABFT following the presumed peak feeding and spawning periods.

Behaviors relevant to ABFT energy balance during the spawning period are also not well understood. Teo et al. (2007*b*) speculated that vertical movements in the Gulf of Mexico during the presumed spawning period reflect thermoregulatory and spawning behaviors, but they were not able to definitively identify feeding and spawning behaviors with electronic tags. The presence of ABFT in the Mediterranean year-round and in the Gulf of Mexico in early winter, well outside of the putative spawning period (Galuardi et al. 2010), suggest that spawning habitat is also productive foraging habitat. Stomach content analyses in the Mediterranean during the putative breeding period are inconclusive, but generally suggest that feeding is substantially reduced, particularly during spawning migrations (see references in Mather et al. 1995). The high energy requirements for ABFT, particularly during migration and spawning, coupled with observations including stomach contents and depth behaviors documented by electronic tags, suggest that considerable feeding occurs during this time.

In our model, we greatly simplified the costs associated with migration and spawning by using the amount of feeding that occurs during migration and spawning and the spawning migration distance. In reality, spawning cost may include additional factors such as increased mortality due to thermoregulatory stress and reduced oxygen or food availability. Regardless, the parameters chosen to represent the cost of migration and spawning, particularly the amount of feeding during migration and spawning, are extremely important for shaping the optimal ABFT life history strategy. Given this result and the complexity of observed ABFT migration behaviors (Galuardi et al. 2010), a topic for future research is to investigate specific migration activities (e.g., feeding, minimizing energy expenditure) and concurrent changes in physiology and condition during migration and spawning. Deployment of electronic tags such as the Daily Diary Tag (see Gleiss et al. 2009) with sensors capable of identifying feeding behavior, spawning, and metabolic rate will be particularly important to this effort. This work would improve our understanding of the processes that determine migration and spawning behavior — critical components of the ABFT life history that link with ABFT resiliency to habitat change and fishing pressures.

Skipped spawning

Deferred spawning after initial maturation is a behavior that represents a trade-off between immediate and anticipated future reproduction (Jørgensen et al. 2006), which has been observed in moderately long-lived fish species (Rideout et al. 2005), but has not been resolved for ABFT (Rooker et al. 2007). The presence of large, presumably mature fish outside of the Gulf of Mexico during the spawning period (Lutcavage et al. 1999; Block et al. 2005; Galuardi et al. 2010) may indicate that some ABFT skip spawning. If ABFT do omit spawning, this would also influence stock assessment and quota-setting calculations (Secor 2007). The simulations suggest that given the cost of migration (distance covered and the proportion of metabolic costs met during migration) and the single-spawning location assumption of the reference scenario, skipped spawning emerges as reproductive strategy that in some cases optimizes life-long fecundity. However, the model suggests that if skipped spawning is to occur, it would be most likely observed in younger fish that have recently matured. This agrees with results from a modeling study that found skipped spawning to be more common among young Atlantic cod (Jørgensen et al. 2006). However, Galuardi et al. (2010) found presumably older, large fish outside of known spawning areas during the period when spawning typically occurs. These fish were either skipping reproduction or spawning at unknown spawning locations. Our results suggest that skipped spawning is not likely to be an important strategy for older, mature fish and that the larger fish observed by Galuardi et al. (2010) away from spawning areas during the spawning period may have been selecting alternative, perhaps lower-quality spawning habitats. Alternatively, the model may be missing a critical process that would otherwise explain skipped spawning in older fish. A firm verdict on the question of skipped spawning in ABFT awaits results from reproductive physiology and maturity studies conducted in both ABFT feeding and spawning grounds.

Differences between eastern and western bluefin tuna

Results from this study also show that conditions that support differences in age-at-maturity generally result in very different growth curves — a pattern that emerges from differing age-specific allocation to reproduction and growth. However, it is also important to recognize that similar growth curves can reflect very different life histories. Restrepo et al. (2010) have recently proposed an improved growth curve for W-BFT that is similar to the currently accepted growth curve for E-BFT. If we accept that fish that spawn primarily in the Mediterranean or Gulf of Mexico mature at different ages, and that despite these differences, E-BFT and W-BFT have similar growth curves, we can consider whether differences in environmental conditions are responsible for this pattern. Although additional combinations may produce the same result, we provide one potential explanation for observed differences in W-BFT and E-BFT life histories; W-BFT experience lower mortality and increased migration and spawning costs

compared with E-BFT. This is in concordance with observations that suggest that spawning grounds in the Gulf of Mexico may be further from the feeding grounds than is the case for the Mediterranean. However, results from electronic tagging and microchemical analysis are inconclusive regarding the relative cost of migration for E-BFT vs. W-BFT. This presents an opportunity for future work in this area. Also, fishing in the Mediterranean has been ongoing for more than 1000 years (Fromentin and Powers 2005), and this higher mortality could have led to life history adaptations. However, mortality exclusively on mature individuals is generally expected to delay maturation (Law and Grey 1989), so a higher mortality would need to also affect juveniles and adolescents if it were to explain the observed differences between E-BFT and W-BFT.

Future model development

The model used in this study is a general one, and its structure could be changed to address additional questions. For example, the current model assumes a single spawning region that limits individuals to natal homing behavior. However, size-class frequency data of Pacific bluefin tuna (Itoh 2006) and ABFT in the Mediterranean (Heinisch et al. 2008) suggests that bluefin tuna may use different spawning sites at different times as they grow (enhanced spawning behavior plasticity). In addition, repeat homing and schooling behavior, where younger individuals learn successful migration behavior from older fish, has also been proposed (Fromentin and Powers 2005). Differences in spawning migration behavior such as these can influence the optimal life history strategy (Jørgensen et al. 2008), so future work could involve expanding the current model structure to include multiple spawning areas and alternative spawning behaviors. In addition, as we learn more about the distribution of habitat that is suitable for development of viable ABFT larvae, variability in the quality of spawning habitats can be incorporated into the modeling structure. In addition, ABFT can use multiple feeding areas (Fromentin and Powers 2005; Sibert et al. 2006; Walli et al. 2009) within years and over longer time periods, so future model development could be used to investigate trade-offs that determine optimal decisions for selecting foraging sites characterized by different seasonality in food quality and availability and distance from spawning habitat. Finally, in recent decades, fishing mortality experienced by E-BFT occurs in both the spawning and feeding areas, while W-BFT experience fishing mortality primarily in the feeding area (Fromentin and Powers 2005). Based on model studies, the location of fishing mortality (spawning vs. feeding grounds) can influence the optimal age-at-maturity and growth for a migratory species (Dunlop et al. 2009). The influence of variability in the location and size structure of fishing mortality can be explored with a simple extension of this model.

For the future, it might also be interesting to make a similar model that would allow other fitness measures. State-dependent dynamic programming models allow rich state dependence but require that the optimization criterion is the same over the course of optimization (in our case, expected lifetime reproductive success) (Houston and McNamara 1999). Alternative fitness measures include the geometric mean population growth and invasion fitness, which would increase the influence of poor years more than our current fitness measure. These fitness measures have advantages particularly if there is frequency dependence, bet hedging, or environmental fluctuations (Metz et al. 1992; Mylius and Diekmann 1995). Using these fitness measures would require changing to a modeling methodology where individual state is less resolved, for example, to the adaptive dynamics framework (Metz et al. 1992) or individual-based model with evolving traits, where explicit population dynamics makes fitness emergent (e.g., Strand et al. 2002).

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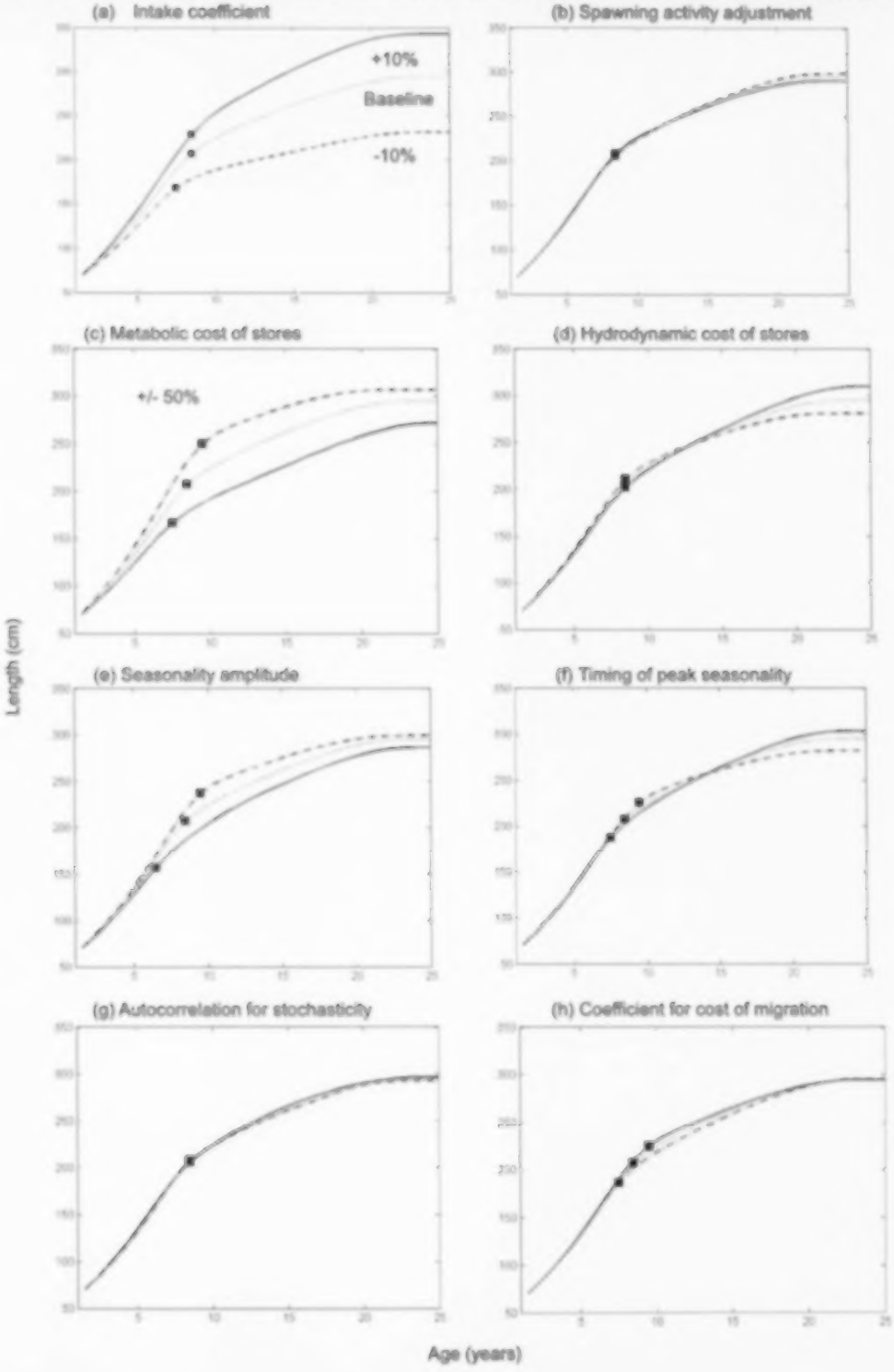
Appendix A

Figure A1 appears on the following page.

Fig. A1. Results from secondary sensitivity analyses. The mean length-at-age from model output when each parameter in Table 3 is varied above (solid line) and below (dot-dashed line) its baseline value (dotted line). The age at 50% maturity for each simulation is indicated by a black square.



Use of genetic markers to aid in re-establishing migratory connectivity in a fragmented metapopulation of bull trout (*Salvelinus confluentus*)

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Abstract: Dams and other barriers fragment important migratory corridors for bull trout (*Salvelinus confluentus*) across the species range. Three dams constructed without fish passage facilities prevented migratory bull trout in the Lake Pend Oreille and Clark Fork River system in Idaho and Montana, USA, from returning to their natal spawning tributaries for nearly 100 years. We genotyped bull trout from 39 spawning tributaries to assemble a baseline data set that we used to develop a real-time genotyping and analysis protocol to assist with upstream fish transport decisions. Self-assignment tests and analysis of blind samples indicated that unknown individuals could be assigned to their region of origin with a high degree of confidence. From 2004 to 2010, genetic assignments were conducted for 259 adult bull trout collected below mainstem dams. Based on genetic assignments, 203 fish were transported upstream above one or more dams. This protocol has helped re-establish connectivity in a fragmented system, providing increased numbers of spawning adults for numerically depressed populations above the dams. We discuss the utility of genetic data for assisting with upstream passage decisions.

Résumé : Des barrages et d'autres barrières fragmentent d'importants corridors de migration de l'omble à tête plate (*Salvelinus confluentus*) sur toute son aire de répartition. Trois barrages construits sans passe migratoire, dans les bassins versants du lac Pend Oreille et de la rivière Clark Fork en Idaho et au Montana, É.-U., ont empêché la migration de retour des ombles à tête plate vers leurs tributaires de naissance pour frayer pendant presque 100 ans. Nous avons déterminé le génotype d'ombles à tête plate de 39 tributaires de fraie afin de construire une banque de données de base que nous avons utilisée pour mettre au point un protocole de détermination et d'analyse des génotypes en temps réel afin de faciliter les décisions de déplacement des poissons vers l'amont. Les tests d'auto-assignation et l'analyse d'échantillons en aveugle indiquent que des individus inconnus peuvent être reliés à leur région d'origine avec un niveau élevé de confiance. De 2004 à 2010, nous avons procédé à des assignations génétiques de 259 ombles à tête plate adultes récoltés en aval des barrages du cours principal. D'après les résultats de ces assignations génétiques, nous avons déplacé 203 poissons vers l'amont au-delà d'un ou plusieurs barrages. Ce protocole a aidé à rétablir la connectivité dans un système fragmenté en procurant un nombre accru d'adultes reproducteurs dans des populations numériquement réduites en amont des barrages. Nous discutons de l'utilité des données génétiques pour faciliter les décisions reliées aux déplacements vers l'amont.

[Traduit par la Rédaction]

Introduction

Riverine systems world-wide have been extensively fragmented by the construction of dams and other barriers (Jackson et al. 2001). The effects of fragmentation on the aquatic community, and specifically fishes, have been well documented and include changes in species assemblages (Guenther and Spacie 2006), disruption of migratory corridors (Neraas and Spruell 2001; Schmetterling 2003), reductions in genetic variation within and reduced gene flow among fragmented populations (Yamamoto et al. 2004; Bessert and Ortí 2008; Reid et al. 2008), and increased risk of extirpation (Morita et al. 2009). Many fish species express resident and migratory life history strategies and rely on large connected rivers to access multiple habitat types (Northcote 1997); therefore, the maintenance of migratory corridors has

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been recognized as an important factor for conserving these species (Rieman and Dunham 2000).

Although the negative implications associated with fragmentation of aquatic habitats are recognized, developing solutions to these problems can be difficult. Often fish passage facilities (e.g., fish ladders) are constructed to allow fish to move upstream of barriers; however, these facilities are often designed to benefit commercially important fishes (e.g., salmonids), and other species may not be able to use them as effectively (Moser et al. 2002; Parsley et al. 2007). If passage facilities do not exist, fish are often collected below barriers and manually transported upstream (Schmetterling 2003). When multiple populations exist above and below barriers, it can be difficult to determine where fish collected in an area of admixture should be transported. Furthermore, it may be undesirable to move individuals from a numerically depressed population below a barrier to areas upstream. When fish passage facilities are lacking, and transporting all fish collected below a barrier upstream is not appropriate, information regarding an individual's population of origin could be useful for making passage decisions.

Genetic data have been widely used to evaluate the effects of fragmentation on fish populations and could also be used to help develop solutions to these problems. Previously, genetic assignment tests have been used to identify the population or strain of origin of unknown individuals for broodstock selection, harvest management, and recruitment estimates (Page et al. 2003; Smith et al. 2005; Matala et al. 2009). Despite obvious applicability, genetic data have not been widely used for making upstream fish transport decisions. In this study, we explore the utility of using genetic population assignments to assist with fish passage decisions for bull trout (*Salvelinus confluentus*).

Bull trout exhibit multiple life history forms, including resident fish that remain in natal tributaries (e.g., headwater streams) as well as migratory fish that utilize larger rivers, lakes, reservoirs, and marine habitats for growth and rearing before returning to natal tributaries to spawn (Rieman and McIntyre 1993; Northcote 1997). Migratory individuals provide a means for recolonization of extirpated populations and geneflow among small populations, enabling persistence in dynamic landscapes and counteracting the loss of fitness caused by inbreeding (Northcote 1997; Rieman and Allendorf 2001). Migratory corridors utilized by bull trout have been extensively fragmented, and many migratory barriers lack adequate fish passage facilities. Presently, bull trout are listed as a threatened species in the United States (US Fish and Wildlife Service 1999), and in Canada bull trout are a species of conservation concern. Habitat fragmentation and loss of migratory individuals are thought to be major factors limiting bull trout recovery (Rieman et al. 1997).

The Lake Pend Oreille and Clark Fork River (LPO–CFR) system in Idaho and Montana (Fig. 1) contains a large bull trout population complex with genetically distinct local spawning populations in several tributaries (Spruell et al. 1999; Neraas and Spruell 2001). Historically, bull trout migrated from tributaries in the Clark Fork River system to Lake Pend Oreille for growth and rearing and then returned to natal tributaries to spawn. The construction of three mainstem dams, however, has fragmented the system for nearly 100 years. Thompson Falls Dam was constructed first in 1913 and was followed by Cabinet Gorge Dam in 1952 and Noxon Rapids Dam in 1959 (Fig. 1). Although fish can still travel downstream through the dams, none of these dams included upstream fish passage facilities when they were constructed. Historical data on bull trout populations in the LPO–CFR system are limited prior to the 1950s, although declines were observed following this time period (Pratt and Huston 1993). Large adult bull trout continue to enter the Clark Fork River from Lake Pend Oreille and congregate downstream from Cabinet Gorge Dam from spring through early autumn. Previous genetic analysis found that bull trout collected below Cabinet Gorge Dam most likely originated from upstream populations (Neraas and Spruell 2001). These authors, as well as an expert panel (Epifanio et al. 2003), concluded that the benefits of passing fish upstream outweighed the potential risks, and the recommendation was made to provide upstream passage at Cabinet Gorge Dam (Epifanio et al. 2003). Based on these recommendations, from 2001 to 2003, up to 35 adult bull trout collected below Cabinet Gorge Dam were passed upstream into Cabinet Gorge Reservoir each year.

Although passing fish above Cabinet Gorge Dam benefits populations immediately above the dam, fish originating above Noxon Rapids and Thompson Falls Dams that migrate downstream to Lake Pend Oreille were still unable to return to natal tributaries. Furthermore, the possibility existed that fish originating from populations below Cabinet Gorge Dam may be inadvertently passed upstream, which could negatively impact depressed populations below the dam. Because of these concerns, there was a need for information on the origins of fish collected below mainstem dams to help guide upstream fish transport decisions. The objective of our study, therefore, was to develop a real-time genetic assignment protocol that could be used to determine the geographic origin (i.e., spawning tributary–population) of bull trout collected below mainstem dams. This information could then be used to help make more informed transport decisions and maximize conservation benefits to a greater number of populations.

Materials and methods

Sample collection

For fisheries management purposes, the LPO–CFR system is divided into four "regions" based on the location of mainstem dams: Region 1 corresponds to the area downstream of Cabinet Gorge Dam and includes tributaries to Lake Pend Oreille and the Pend Oreille River; Region 2 corresponds to the reservoir and tributaries of the Clark Fork River between Cabinet Gorge and Noxon Rapids Dams; Region 3 corresponds to the reservoir and tributaries between Noxon Rapids and Thompson Falls Dams; and Region 4 corresponds to the Clark Fork River and its tributaries above Thompson Falls Dam. Bull trout were collected from 39 tributaries from all four regions within the LPO–CFR system (Table 1; Fig. 1) to develop a baseline data set for population assignments. Previous studies found that most migratory bull trout migrate downstream from natal rearing areas to larger bodies of water at ages 2 or 3 (Fraley and Shepard 1989; Mogen and Kaeding 2005; Downs et al. 2006), so sampling targeted juvenile fish between 75 and 250 mm in total length to increase the likelihood of collecting fish in their natal tributaries. Juve-

Fig. 1. Map of the tributaries in the Lake Pend Oreille and Clark Fork River system that were sampled for this study. Numbers on the map correspond to spawning tributary ID numbers in Table 1. Region 1 corresponds to Lake Pend Oreille and the Clark Fork River downstream of Cabinet Gorge Dam; Region 2 corresponds to the area between Cabinet Gorge and Noxon Rapids Dams; Region 3 corresponds to the area between Noxon Rapids and Thompson Falls Dam; and Region 4 corresponds to the area upstream of Thompson Falls Dam.

niles were primarily collected by stream electrofishing, but in 2001 and 2002, juvenile bull trout were collected primarily by weir traps in Rock Creek, East Fork Bull River, and Graves Creek. When stream electrofishing was used, at least two locations were sampled in each tributary, and sampling locations were dispersed throughout the drainage to avoid sampling large numbers of related individuals. Sampling efforts also targeted fish from multiple age classes as an additional means to reduce the likelihood of collecting large numbers of related individuals. Twenty-five of the 39 tributaries were sampled during multiple years (Table 1) to increase sample sizes and to account for any changes in population allele frequencies over time. Fin clips were taken from all juvenile bull trout collected and preserved in 100% non-denatured ethanol.

In 2007, 62 "blind" samples of juvenile bull trout from tributaries throughout the study area were collected to provide an estimate of our ability to accurately assign unknown origin fish to their collection location. These individuals were collected independently of baseline sample collections to perform cross-validation of assignment techniques and avoid biasing our assessment of assignment success (i.e., "high grading"; Anderson 2010). The original collection code and collection location of each individual was kept confidential from personnel responsible for laboratory analysis and conducting genetic assignments until after these individuals were analyzed.

Adult bull trout greater than 300 mm total length were captured below Cabinet Gorge Dam by boat electrofishing, angling, and a hatchery fish ladder trap from April through October 2001 to 2003. A portion of these fish were part of an experimental fish transport program and were tagged and released upstream between Cabinet Gorge Dam and Noxon Rapids Dam. Fin clips were taken from 65 of these fish so that genetic assignment techniques could be used to determine their geographic origin.

Laboratory analysis

DNA was extracted from all baseline samples, blind samples, and adults collected below Cabinet Gorge Dam using a

Table 1. Collection information, results of Hardy–Weinberg equilibrium (HWE) and linkage equilibrium tests (LD), F_{IS} estimates, and mean population identity estimates (I) and associated P values for 39 bull trout collections from the Lake Pend Oreille – Clark Fork River (LPO–CFR) system.

Tributary collection No.	Tributary name	Years sampled	No. of samples	HWE-1	HWE-2	LD	F_{IS}	I	P
1	Middle Fork East River	2002, 2003	86	7	1	26	0.044	0.270	0.822
2	Uleda Creek	2003, 2005	67	11	10	55	0.019	0.395	0.999
3	Indian Creek	2003	18	3	2	0	0.009	0.504	0.788
4	Gold Creek	2003	44	7	6	17	–0.044	0.418	0.952
5	Upper Priest River	2003	49	5	2	10	–0.029	0.284	0.002
6	Char Creek	UM	29	2	0	1	–0.053	0.389	0.213
7	East Fork Lightning Creek	UM, 2005, 2007	139	3	1	10	0.020	0.312	0.852
8	Gold Creek	UM, 2005	73	1	0	1	0.002	0.234	0.404
9	Granite Creek	UM, 2005	94	2	1	4	0.010	0.304	0.721
10	Grouse Creek	UM, 2007	70	2	1	2	0.014	0.401	0.970
11	Lightning Creek	UM	22	2	2	0	0.018	0.332	0.140
12	Morris Creek	UM, 2007	32	1	0	3	0.004	0.362	0.944
13	Porcupine Creek	UM	38	3	0	2	0.034	0.348	0.960
14	Savage Creek	UM	40	1	1	5	–0.038	0.348	0.925
15	Trestle Creek	UM, 2005	156	2	0	3	0.018	0.321	0.962
16	Rattle Creek	UM, 2005	78	5	2	17	–0.004	0.294	0.137
17	Johnson Creek	2006	47	4	1	14	–0.081	0.388	1.000
18	Twin Creek	UM, 2005	59	10	9	66	–0.059	0.351	0.998
Cabinet Gorge Dam									
19	East Fork Bull River	UM, 2001, 2007	95	6	2	14	0.021	0.269	0.562
20	Rock Creek	UM, 2001, 2003	80	7	3	22	0.017	0.265	0.539
Noxon Rapids Dam									
21	Swamp Creek	UM, 2006, 2007	35	10	3	9	–0.123	0.451	0.624
22	Graves Creek	UM, 2002, 2008	67	5	1	19	0.024	0.384	0.839
23	Prospect Creek	UM, 2003, 2007	150	10	10	43	0.045	0.258	0.304
24	Cooper Gulch	2007	41	9	6	16	0.013	0.280	0.959
25	Vermilion River	2004	42	2	1	7	–0.062	0.418	0.199
Thompson Falls Dam									
26	Thompson River	UM, 2005	71	5	4	5	0.092	0.377	0.919
27	Fishtrap Creek	UM, 2006, 2007	85	2	0	7	–0.006	0.324	0.087
28	Cedar Creek	2005	25	3	1	0	0.014	0.334	0.626
29	Albert Creek	2007	48	1	0	1	–0.015	0.525	0.149
30	Fish Creek	2004, 2005	56	10	9	46	0.012	0.259	0.999
31	Little Joe Creek	2005, 2007	58	6	1	3	0.065	0.363	0.908
32	Copper Creek	UM	50	2	0	1	–0.027	0.385	0.957
33	Monture Creek	UM	36	2	0	1	0.013	0.368	0.374
34	North Fork Blackfoot River	UM	39	4	0	1	0.067	0.314	0.958
35	Rattlesnake Creek	UM	27	2	0	2	0.019	0.295	0.041
36	Meadow Creek	UM	27	3	0	4	–0.086	0.391	0.890
37	North Fork Jocko River	2004, 2005, 2006	33	3	1	1	–0.048	0.386	0.418
38	South Fork Jocko River	2004, 2005, 2006	63	8	5	50	0.030	0.357	0.988
39	Upper Rock Creek	2003	29	1	0	0	0.046	0.287	0.242

Note: Tributary numbers correspond to numbers in Fig. 1. UM in the years sampled column refers to samples collected from the University of Montana archives for which specific collection years were not available. HWE-1 represents the number of loci (out of 12) deviating from HWE before Bonferroni correction. HWE-2 represents the number of loci (out of 12) deviating from HWE following Bonferroni correction.

Chelex 100 (Sigma Chemical Co.) resin solution as described by Miller and Kapuscinski (1996). All samples were then genotyped at a suite of 12 microsatellite loci: *Omm1070*, *Omm1128*, *Omm1130* (Rexroad et al. 2001); *Sco104*, *Sco105*, *Sco106*, *Sco107* (K. Warheit and S. Young, Washington Deptartment of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98503, USA, personal communication, 2011); *Sco200*, *Sco212*, *Sco216*, *Sco218* (DeHaan and Ardren 2005); and *Smm22* (Crane et al. 2004). PCR reactions were carried out in 15 µl volumes containing 1× PCR buffer (10 mmol·L^{-1} Tris–HCl, 50 mmol·L^{-1} KCl, 1% Triton X-100), 0.2 mmol·L^{-1} dNTPs, 0.5 µmol·L^{-1} forward and reverse primer, 1.5 or 2.0 mmol·L^{-1} $MgCl_2$, and 0.2 units of *Taq* DNA polymerase (Promega Co.). Following PCR amplifica-

tion, reactions were pooled and automated electrophoresis was carried out on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Inc.) using the GeneScan-500LIZ size standard (Applied Biosystems, Inc.). Electropherograms were analyzed and genotypes were determined for each individual at each locus using the program GENEMAPPER v. 4.0 (Applied Biosystems, Inc.). Hybrids between bull trout and non-native brook trout (*Salvelinus fontinalis*) have been observed in the Clark Fork River system (Kanda et al. 2002). Several of the 12 loci we used show diagnostic differences between the two species (DeHaan et al. 2010), and any hybrids we detected were removed from further analysis.

Analysis of the baseline data set and assignment techniques

Baseline samples were grouped according to their tributary of collection for statistical analysis. When samples have been collected over multiple years, it may make sense to pool collections from the same location (i.e., spawning tributaries) to provide the most accurate estimate of allele frequencies (Waples 1989). However, there may be genetic differences among temporal replicate samples (Østergaard et al. 2003; Walter et al. 2009; Palstra and Ruzzante 2010), particularly in small populations with low effective size. We conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to evaluate the level of genetic variation among tributary collections due to temporal sampling. AMOVA was conducted using the program ARLEQUIN v. 3.11 (Excoffier et al. 2005) and included temporal replicates from the 25 tributaries that had been sampled multiple times. Based on AMOVA results (see below), we combined temporal replicates from the same tributary for subsequent statistical analysis and genetic assignment purposes.

Collections from each tributary were tested for conformance to Hardy–Weinberg equilibrium (HWE) expectations using exact tests implemented in the program GENEPOP v. 4.0.7 (Raymond and Rousset 1995). GENEPOP was also used to test each collection for evidence of linkage disequilibrium. Significance values for HWE and linkage disequilibrium tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). We estimated F_{IS} (Weir and Cockerham 1984) values for each tributary collection using GENEPOP. Because collections of juvenile salmonids may contain groups of closely related individuals (Hansen et al. 1997), we also estimated the mean pairwise identity (I) for each tributary and compared this with its expected value based on 1000 permutations of the data set using the program IDENTIX (Belkhir et al. 2002). P values were determined as the proportion of permutations that exceeded the mean I value.

Individual tributaries often contain genetically distinct spawning populations of bull trout (Spruell et al. 1999; Whiteley et al. 2006), but in some cases, bull trout from multiple tributaries may represent a single genetic group (Warnock et al. 2010). A nonspatial Bayesian clustering analysis was conducted using BAPS v. 5.3 (Corander et al. 2008) to determine the most appropriate number of genetic units for population assignment purposes. This method treats the microsatellite allele frequencies and the number of genetically diverged populations as random variables. We chose to cluster groups of individuals, so BAPS used the tributary of origin for each sampled individual as a prior in a genetic mixture model. Population clusters (K) were identified that resulted in groups of tributaries that resembled each other as much as possible based on allele frequencies. The posterior mode distribution was estimated for all potential combinations of K from 1 to 50 using the stochastic optimization algorithm of Corander et al. (2006). The K with the highest global optimum, as measured by logml values (natural logarithm of the marginal likelihood of the data), was identified as the optimal partition of population clusters. A detailed description of the Bayesian model used in BAPS can be found in Corander and Marttinen (2006). Clusters of tributaries identified by BAPS were re-analyzed independently to identify further substructuring.

When developing baseline data sets for population assignment purposes, it may not be possible to sample all of the populations within the study area, which can be problematic when individuals for assignment originate from unsampled populations. If unsampled populations are genetically similar to geographically proximate populations that have been sampled, unknown individuals from unsampled populations would presumably be assigned to a neighboring population. To infer the genetic relationship between spawning tributaries, we generated a consensus neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distances between all population pairs using the program PHYLIP v. 3.6 (Felsenstein 2005). The bootstrap procedure was used to generate 1000 replicate data sets based on observed allele frequencies. We also estimated the level of genetic variation among tributaries (pairwise F_{ST}) and then tested for isolation by distance with a Mantel test in GENEPOP (1000 permutations) comparing the natural log of geographic distance in river kilometres between tributaries with the pairwise genetic distance between tributaries measured as $F_{ST}/(1 - F_{ST})$. Geographic distance was measured as the fluvial distance between river mouths (Appendix A, Table A1). Distances between tributaries separated by Lake Pend Oreille were calculated by determining the most direct path of migration through the lake.

Tributary collections were grouped according to clusters identified by BAPS for population assignment purposes. We then performed leave-one-out assignment tests of our baseline data set using the program WHICHRUN v. 4.0 (Banks and Eichert 2000) to estimate the ability of our baseline data set to assign individuals to the tributary–genetic group they were collected from. With this procedure each individual fish was removed from the baseline data set and treated as an unknown origin individual. The allele frequencies for each tributary–genetic group were then recalculated without that individual, and the individual was assigned to its most likely tributary–genetic group of origin. Likelihood values for genetic assignments were calculated using the methods of Banks and Eichert (2000). The number of individuals assigned to the tributary they were collected from provides a means of estimating the ability of the baseline data set to assign unknown individuals. We used the log of odds (LOD) ratio between the first and second most likely tributary–genetic group of origin to assess the confidence in genetic assignments (Banks and Eichert 2000).

To allow for gene flow among tributaries and to decrease the chance of transporting fish to the incorrect location, upstream transport decisions in the LPO–CFR focus on return-

ing fish to the correct management region of origin rather than to specific tributaries. Because upstream transport focuses on the different management regions, we determined the number of individuals in the leave-one-out analysis that were assigned to a tributary–genetic group in the management region they were collected from. For example, if an individual was collected in East Fork Bull River (population 19) in Region 2 but was assigned to Rock Creek (population 20) also in Region 2, we considered this individual to be assigned within the correct region. We examined the LOD values for fish assigned to tributary–genetic groups outside the region they were collected from ("incorrect" assignments) and fish assigned to tributary–genetic groups within the region they were collected from ("correct" assignments) to assess confidence in the genetic assignments and to determine if there was an appropriate critical LOD value for upstream transport decisions.

We then assigned the 62 blind samples and the 65 fish collected at Cabinet Gorge Dam from 2001 to 2003 to their most likely tributary–genetic group and the corresponding region of origin within the LPO–CFR system using the methods described above. Once genetic assignments had been made for the blind samples, the true collection locations were compared with the genetic assignments to evaluate the accuracy of the baseline data set for assigning individuals to their tributary–genetic group of origin and assigning individuals within their region of origin. Comparisons between genetic assignments and the true collection locations and the LOD values for the blind samples were used to evaluate our ability to assign fish to the tributary–genetic group and management region they were collected from.

Real-time genotyping and analysis of unknown origin adults

Using our baseline genetic data set, we developed a real-time genotyping and analysis protocol to facilitate upstream passage for adult bull trout in the Clark Fork River. Biologists collected adult bull trout below Cabinet Gorge Dam by boat electrofishing, angling, and at a hatchery fish ladder from April through October 2004 to 2010. Captured fish were fin-clipped, passive integrated transponder (PIT)-tagged, and held in a streamside holding facility during sample processing.

Upon receipt of fin clips (typically within 36–40 h of collection), DNA was extracted and samples were genotyped at 12 microsatellite loci following the procedures described above. Adult bull trout were assigned to their most likely tributary–genetic group of origin and the associated region within the LPO–CFR system using WHICHRUN, and LOD values were used to evaluate the confidence of genetic assignments. The baseline data set used for genetic assignments was updated each year as new tributary collections were added. Based on genetic assignments, adult bull trout were either transported upstream to the appropriate management region of the Clark Fork River or released below Cabinet Gorge Dam (fish assigned to tributaries within Region 1). Fish transported upstream were primarily released in the mainstem Clark Fork River and not in their most likely natal tributary to allow for gene flow among tributaries within the same region. Individuals genetically identified as hybrids were removed from the system.

Results

Analysis of the baseline data set and assignment techniques

A total of 2298 baseline samples were collected and the number of individuals collected from each tributary ranged from 18 (Indian Creek) to 156 (Trestle Creek; Table 1). Results of the temporal replicate AMOVA indicated that 2.41% of the total genetic variation was due to differences among temporal sampling replicates (Φ_{SC} (the level of variation among temporal replicates) = 0.027), whereas 10.99% of the total variation was due to differences among spawning tributaries (Φ_{CT} (the level of variation among tributaries) = 0.110). All values were found to be significantly different from 0.0 ($P < 0.0001$). Because we observed a greater level of variation among tributary collections than we observed among temporal replicate samples, we combined temporal replicates collected from the same tributary for subsequent statistical analysis. Prior to Bonferroni correction, all tributary collections had at least one locus that deviated from HWE (Table 1). Following Bonferroni correction, 26 of the 39 tributaries analyzed had at least one locus that deviated from HWE (Table 1). Although the majority of the tributaries with deviations from HWE had only one or two loci out of equilibrium, a number of tributaries deviated from HWE at the majority of the loci analyzed (Table 1). These same tributaries also had high numbers of locus pairs that showed evidence of linkage (Table 1). Mean pairwise identity (I) values for each tributary collection ranged from 0.234 to 0.525 (Table 1). When we examined the statistical significance of I values, only P values for Upper Priest River (tributary 5) and Rattlesnake Creek (tributary 25) were less than 0.05, suggesting nonrandom sampling of related individuals from these two tributaries.

BAPS identified $K = 33$ as the optimal number of genetic groups in the baseline data set (logml = –105,979; $P = 0.961$). The majority of tributaries grouped independently in the analysis. The exceptions were Char Creek, East Fork Lightning Creek, Morris Creek, and Savage Creek, which grouped together; Lightning Creek, Porcupine Creek, and Rattle Creek, which grouped together; and Prospect Creek and Cooper Gulch, which grouped together. These groupings represent tributaries within the same watershed, and the first two groups are both within the Lightning Creek drainage. Subsequent analysis of these clusters identified nearly all of these tributaries as independent genetic groups with the exception of two clusters: East Fork Lightning Creek and Savage Creek; and Lightning Creek and Porcupine Creek. Pairwise estimates of variation among tributaries that clustered together were relatively low as well (Appendix A, Table A1). Because tributaries that BAPS clustered together were within the same management region and transport decisions focus on returning fish to their region of origin, we chose to use the initial 33 genetic clusters identified by BAPS for genetic assignment purposes.

Geographically proximate tributaries from the same management region tended to group together on the neighbor-joining tree with a few notable exceptions (Fig. 2). Populations from the Priest Lake – Pend Oreille River system (tributaries 1–5) grouped separately from the other Region 1 tributaries. Tributaries from Region 3 grouped with clusters

of tributaries from Region 4 rather than grouping together in a separate cluster, and Fishtrap Creek (tributary 27, Region 4) grouped with the two tributaries from Region 2. Pairwise estimates of genetic variation ranged from 0.022 to 0.385 (Appendix A, Table A1). In general, pairwise estimates of variation were greatest among tributaries further apart geographically (i.e., populations in different regions) and smaller among geographically proximate tributaries. All pairwise estimates were found to be significantly different from 0.0 following Bonferroni correction. We observed a significant isolation by distance relationship ($r = 0.487$; Mantel test $P < 0.00001$; Fig. 3).

Overall the leave-one-out assignment procedure assigned 89.7% of the baseline individuals to the tributary–genetic cluster they were collected from. The proportion of fish assigned to their tributary–genetic cluster of collection ranged from 0.687 to 1.000 and was greater than 0.800 for 28 of the 33 tributary–genetic clusters (Fig. 4; Appendix A, Table A2). Most individuals assigned outside the tributary they were collected from were assigned to geographically proximate tributaries within the same management region (Appendix A, Table A2). Several individuals collected from tributaries immediately downstream of Noxon Rapids and Thompson Falls Dams (tributaries 18, 23, and 24) were assigned to tributaries upstream of the dams. Nearly all of the baseline individuals (96.6%) were assigned to tributaries within the management region where they were collected. The proportion of baseline individuals assigned to tributaries within their region of collection ranged from 0.874 for individuals collected in Region 2 to 0.997 for individuals collected in Region 4 (Table 2). The LOD values for individuals assigned to tributary–genetic groups outside the region where they were collected ($n = 79$) ranged from 0.007 to 9.623, and the LOD values for individuals assigned to tributary–genetic groups within their region of collection ($n = 2219$) ranged from 0.000 to 17.639 (Fig. 5*a*).

Forty-nine of the 62 (79.0%) blind samples were assigned to the tributary–genetic group where they were collected, and 54 of the 62 (87.1%) blind samples were assigned to tributary–genetic groups within the region where they were collected. LOD values for blind samples assigned to tributaries outside their region of collection ranged from 0.048 to 3.043, and LOD values for blind samples assigned to tributaries within their region of collection ranged from 0.136 to 13.488 (Fig. 5*b*). The 65 bull trout collected below Cabinet Gorge Dam from 2001 to 2003 were assigned to tributaries in all four regions of the Clark Fork River each year (Fig. 6*a*), and LOD values for these assignments ranged from 0.004 to 9.408 (Fig. 5*c*).

Real-time genotyping and analysis of unknown adults

From 2004 to 2010, a total of 259 unique adult bull trout collected below Cabinet Gorge Dam were assigned to tributary–genetic group of origin. LOD values for these assignments ranged from 0.020 to 13.702 (Fig. 5*c*). Bull trout collected at the dam were assigned to tributary–genetic groups within all four regions of the LPO–CFR system during each of the 7 years (Fig. 6*a*). Sixteen of these fish were recaptured at Cabinet Gorge Dam in subsequent years. These fish were transported or released based on previous analysis but not re-analyzed. The majority of the samples processed were analyzed within 24 h of receiving fin clips. From 2004 to 2010, a total of 203 adult bull trout were transported to Regions 2, 3, and 4 based on genetic assignments (Fig. 6*b*). In 2004, nearly all fish assigned to upstream regions were transported to Region 2. Fish from Regions 3 and 4 were radio-tagged and transported to Region 2 during 2004 to monitor their movements and select potential sites for a fish trap below Noxon Dam. In 2005 and 2006, fish assigned to Region 2 were transported to Region 2 and fish assigned to Regions 3 and 4 were radio-tagged and transported to Region 3 to monitor their movements and select potential sites for a fish ladder below Thompson Falls Dam. From 2007 to 2010, fish were transported to Regions 2, 3, and 4 based on their genetic assignments. Two individuals collected at Cabinet Gorge Dam were identified as hybrids based on phenotypic characteristics, and genetic data confirmed hybrid ancestry of both fish.

Discussion

Genetic data have been widely used to document the effects of fragmentation due to dam construction on fish populations (Yamamoto et al. 2004; Bessert and Ortí 2008; Reid et al. 2008); however, genetic data have not been widely utilized to help develop solutions to these problems when fish passage structures do not exist. In this study we provide an example of how genetic data can be utilized to assist fish transport decisions when factors such as multiple dams and uncertain origins of the fish collected below dams complicate fish passage. Using the methods we developed, we were able to provide real-time genetic assignments for 259 adult bull trout collected below Cabinet Gorge Dam, which were used to guide upstream transport decisions.

Analysis of the baseline data set and assignment techniques

Studies of salmonid genetics often rely on collections of premigratory juvenile samples collected in natal tributaries to increase the likelihood that individuals originated in their tributary of collection. One problem with this approach is the possibility that collections include large numbers of related individuals (Hansen et al. 1997), which can cause populations to deviate from HWE expectations. Departures from HWE and relatively low estimates of effective numbers of breeding adults from LPO–CFR tributaries (Ardren et al. 2011) are consistent with demographic estimates of small population size based on redd counts (Rieman and Myers 1997; J.M. DosSantos, unpublished data). Departures from HWE have been previously observed in studies of small salmonid populations (Castric et al. 2002; Østergaard et al. 2003), presumably because few adults spawn annually and juvenile collections represent a small number of families or cohorts. Permutation tests suggested that mean pairwise identity values we observed were no higher than expected in most cases.

Results of leave-one-out assignment tests and analysis of the blind samples indicated that we had greater accuracy at assigning fish to their region of origin as opposed to their tributary–genetic group of origin. The majority of the baseline individuals and blind samples assigned to tributary–genetic clusters they were not collected from were assigned to

Fig. 2. Consensus neighbor-joining tree based on Cavalli-Sforza and Edwards' (1967) chord distance for 39 Clark Fork River bull trout spawning tributaries. Values at the nodes represent the percentage of 1000 bootstrap replicates that showed the displayed topology. Only bootstrap values greater than 50.0% are shown.

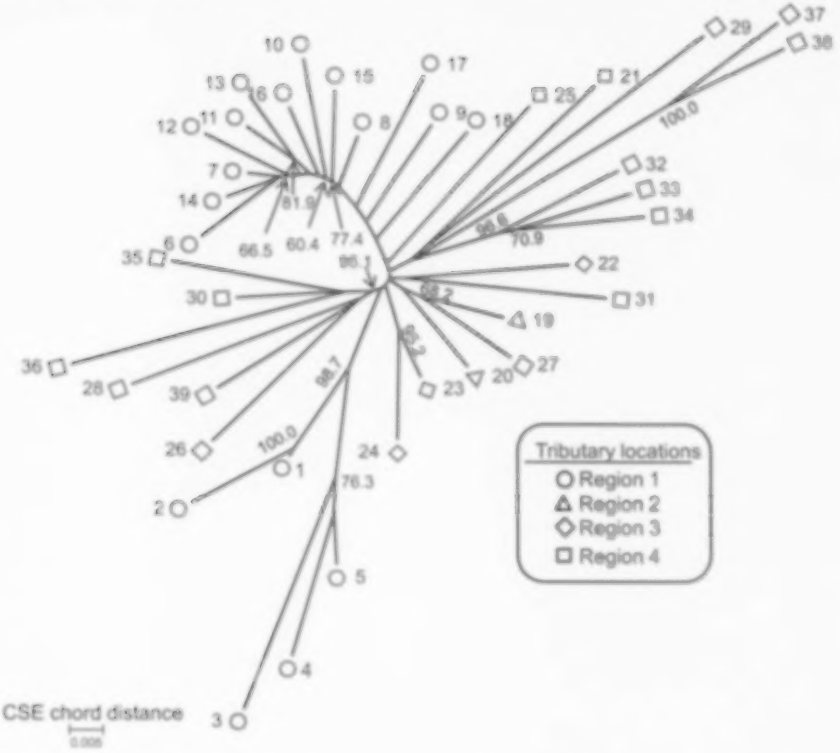
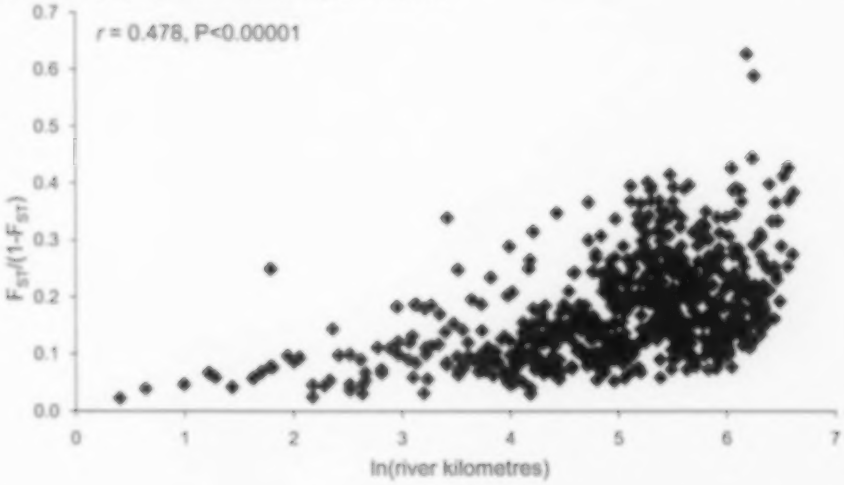
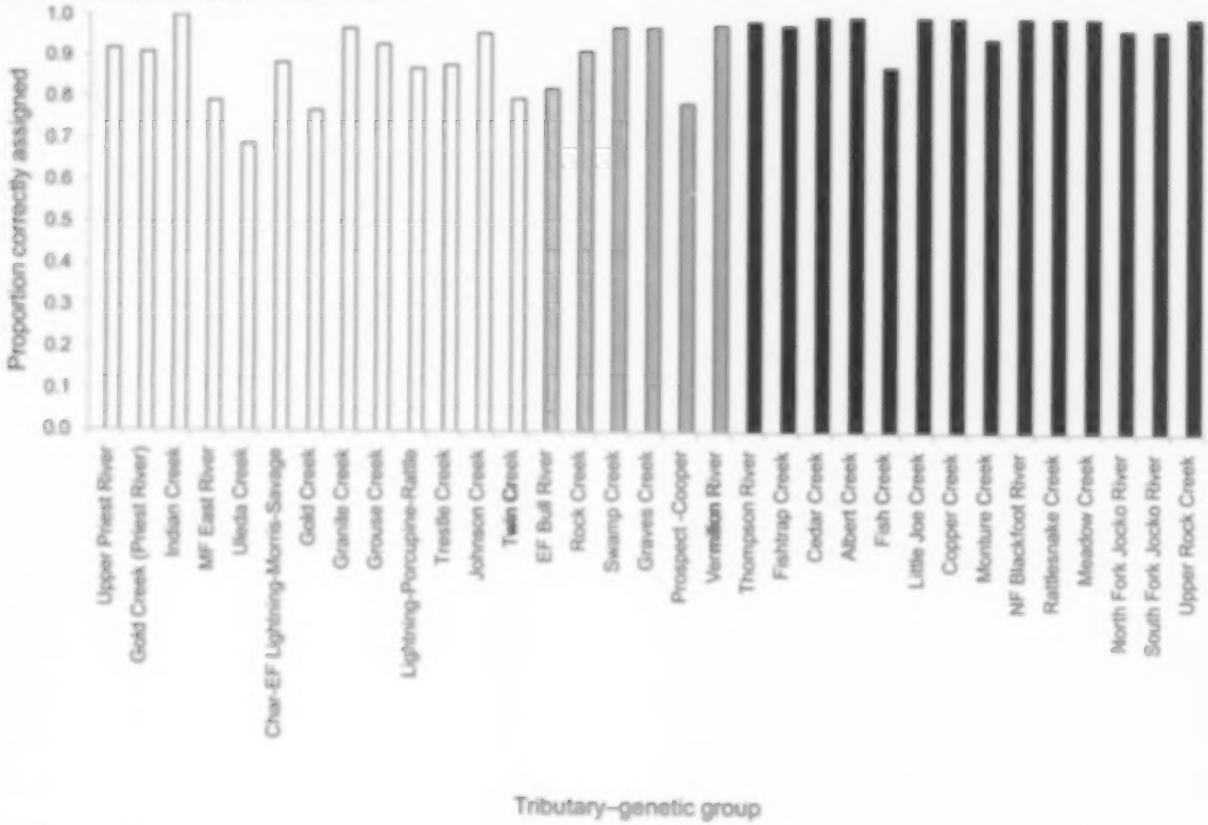


Fig. 3. Analysis of isolation by distance for Lake Pend Oreille – Clark Fork River bull trout populations. Geographic distance was measured as the natural log of the fluvial distance between tributaries (measured in km), and genetic distance was measured as $F_{ST}/(1 - F_{ST})$. A Mantel test indicated that there was a highly significant relationship ($P < 0.0001$).



nearby tributaries within the same management region. This presumably reflects migration and genetic exchange among geographically proximate tributaries within the same region. When individuals are sorted back to their population or strain of origin for management purposes, such as broodstock selection or fish transport, gene flow among populations may be reduced since natural migration among populations is prevented. Gene flow naturally occurs among populations of

Fig. 4. Proportion of individuals collected from each tributary–genetic group assigned to their collection location in the leave-one-out assignment tests. Open bars represent Region 1 tributaries, hatched-line bars represent Region 2 tributaries, grey bars represent Region 3 tributaries, and black bars represent Region 4 tributaries.



bull trout and other migratory salmonids and is important for maintaining genetic variation and adequate effective population sizes (Rieman and Dunham 2000; Rieman and Allendorf 2001). By focusing transport decisions on the different regions of the LPO–CFR system rather than the individual tributaries, we increase the likelihood that individuals are transported to the correct location and still allow for gene flow among tributaries. It is important that fish passage programs that utilize genetic data to make transport decisions recognize the importance of gene flow and allow for genetic exchange among populations.

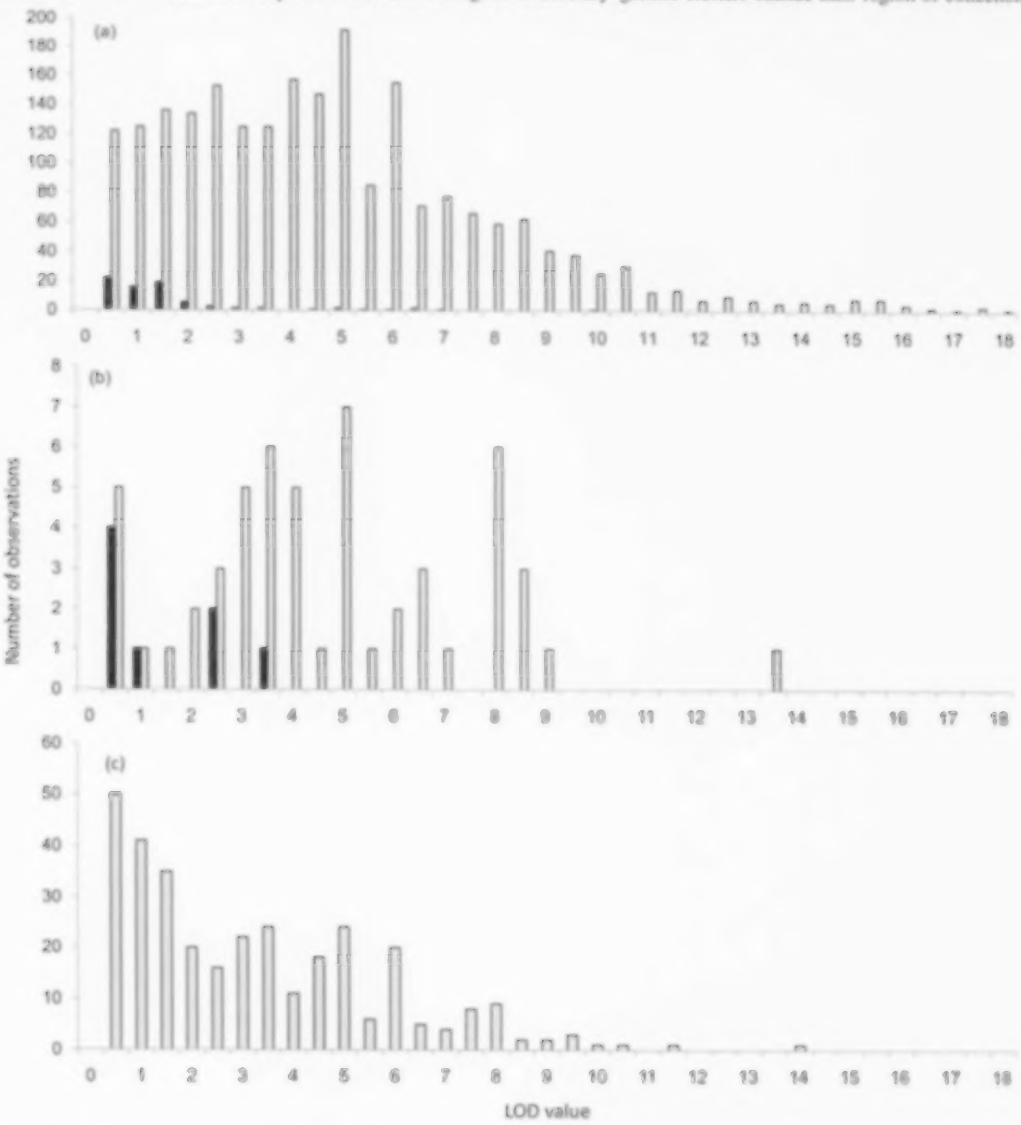
Previously, threshold assignment criteria have been used to increase the confidence in genetic assignments when genetic data are used to sort individuals to their population or strain of origin for management purposes. Matala et al. (2009) found that by using a threshold assignment value, they had 95% confidence in distinguishing among run types of steelhead trout (*Oncorhynchus mykiss*) for hatchery broodstock selection. The use of a threshold LOD value could be used to increase assignment accuracy for LPO–CFR bull trout as well. For instance, if only individual assignments with an LOD value of 1.0 or greater are considered, 97.4% of the individuals in the leave-one-out test and 92.5% of the blind samples were assigned within their region of collection. Although this method could increase the certainty of genetic assignments and the likelihood that fish are returned to their correct region of origin, a number of correctly assigned fish had LOD values below 1.0, and it's difficult to determine the management alternative to passage for these fish. For example, if a fish is assigned to Region 4 with an LOD value less than 1.0, should it be returned to the Clark Fork River below Cabinet Gorge Dam? Examining LOD values does provide a means of evaluating the confidence of genetic assignments, with higher values representing fish that are more likely to be correctly assigned. In this case, however, we chose not to establish threshold assignment criteria for mak-

Table 2. Proportion of baseline individuals assigned to tributary–genetic groups in each management region in the leave-one-out assignment test.

	Assigned to:			
Collected from:	Region 1	Region 2	Region 3	Region 4
Region 1	**0.981**	0.001	0.006	0.012
Region 2	0.006	**0.874**	0.029	0.091
Region 3	0.003	0.006	**0.901**	0.090
Region 4	0.002	0.002	0.000	**0.997**

Note: The proportion of individuals assigned to tributary–genetic groups in the region where they were collected is shown in bold on the diagonal.

Fig. 5. Distribution of LOD values for genetic assignments of baseline fish during leave-one-out analysis (*a*), blind samples (*b*), and unknown origin fish collected at Cabinet Gorge Dam from 2001 to 2010 (*c*). Grey bars represent individuals assigned to tributary–genetic clusters within their region of collection, and black bars represent individuals assigned to tributary–genetic clusters outside their region of collection.

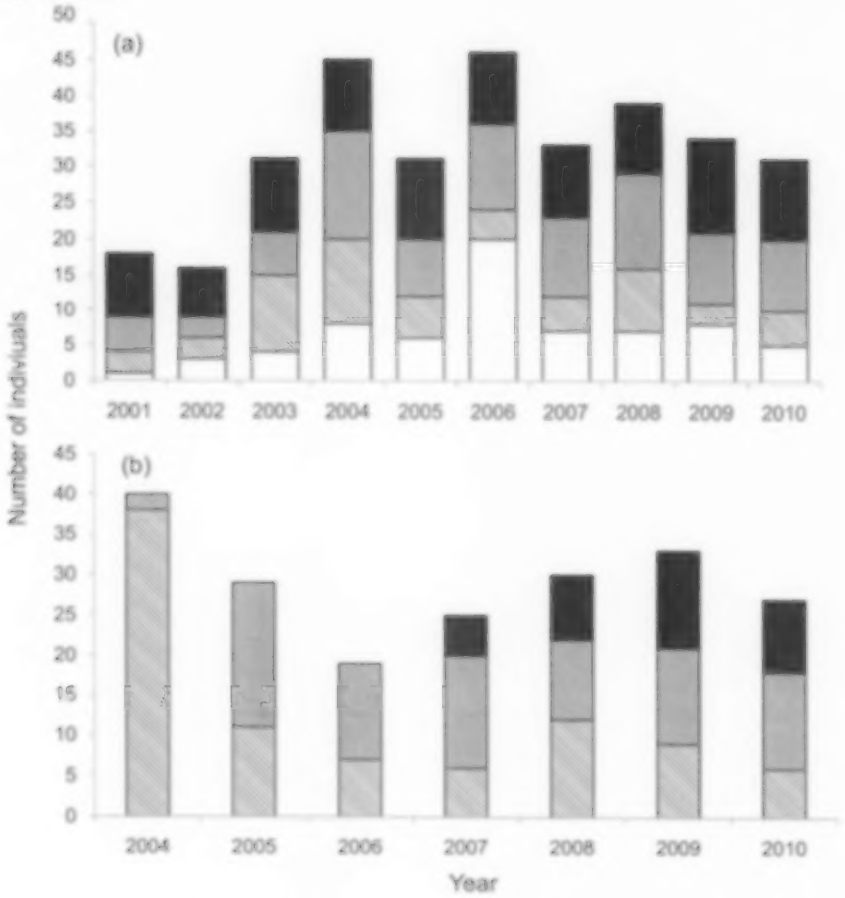


ing transport decisions but to base decisions on the most likely assignment and transport fish accordingly. The use of alternate assignment methods (e.g., Piry et al. 2004) may also provide increased confidence in genetic assignments. Preliminary analysis suggest that for the LPO–CFR system, alternate genetic assignment techniques are more accurate in some cases (see online Supplemental Materials[1]), and we plan to explore these methods further in the future.

When establishing a baseline data set for conducting genetic assignments, it may not be possible to sample all of the baseline populations within the study area. Salmonid populations are often structured as a metapopulation where gene flow occurs among geographically proximate local populations (Rieman and Dunham 2000) and populations typically exhibit an isolation by distance relationship where genetic differentiation among local populations increases with increasing geographic distance (Taylor et al. 2003; Whiteley et al. 2006). Significant isolation by distance observed for LPO–CFR bull trout as well as the neighbor-joining tree and the pairwise estimates of variation suggest that LPO–CFR bull trout are structured in this manner. This has important implications for genetic assignments; if individuals collected

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-098.

Fig. 6. Number of bull trout collected below Cabinet Gorge Dam genetically assigned to tributary–genetic clusters within each region of the LPO–CFR system from 2001 to 2010 (*a*) and the number of fish collected below Cabinet Gorge Dam transported upstream to each region of the Clark Fork River based on genetic assignments from 2004 to 2010 (*b*). Open bar segments represent fish assigned to Region 1, diagonal hatching represents fish assigned and transported to Region 2, grey shading represents fish assigned and transported to Region 3, and black shading represents fish assigned and transported to Region 4. Fish collected from 2001 to 2003 were all transported to Region 2 as part of an experimental fish transport program.



at Cabinet Gorge Dam originate in tributaries that are not part of the baseline data set, they would still presumably be assigned within their region of origin. Since fish transported upstream are released within the mainstem Clark Fork River as opposed to specific tributaries, these individuals could still return to their natal tributaries to spawn.

Real-time genotyping and analysis of unknown adults

Between 2004 and 2010, 203 adult bull trout were transported upstream into Regions 2, 3, and 4 of the Clark Fork River based on their genetic assignments. In the absence of genetic data, 114 of these fish that assigned to tributaries in Regions 3 and 4 would have been transported above Cabinet Gorge Dam but would have still been unable to return to their natal tributaries. Dams and other barriers often separate spawning populations that historically were genetically distinct (Costello et al. 2003; Taylor et al. 2003; Whiteley et al. 2006), and passing all individuals into the same area of the river could result in the homogenization of genetically distinct populations and loss of local adaptations and reduced fitness through outbreeding depression (e.g., Gharrett et al. 1999; Goldberg et al. 2005). Without the use of genetic data, 56 fish that originated in tributaries below Cabinet Gorge Dam would have been transported upstream. Clearly, the use of genetic assignments for making upstream transport decisions in the LPO–CFR system benefits not only the populations above the dam(s) that have been losing migratory fish for several decades but also prevents the upstream passage of fish from downstream populations that may be demographically depressed as well.

Incorporating genetic data into upstream transport programs offers advantages beyond returning fish to their tributary or region of origin. Maintaining a migratory life history type has been recognized as an important factor for the persistence of many salmonid populations (Northcote 1997; Rieman and Dunham 2000). Data on the origins of fish collected below dams can be important for determining how many migratory individuals originate from different populations and

prioritizing tributaries and populations that produce greater numbers of migratory adults for conservation measures, such as the removal of culverts and other in-stream migratory barriers. Genetic data are also important if fish targeted for upstream transport have been observed to hybridize with non-native species. Bull trout have been observed to hybridize with brook trout in the Clark Fork River (Kanda et al. 2002), and we used our markers to screen all individuals targeted for upstream passage for hybrid ancestry as well. Genetic data may be used to prevent hybridized individuals from being transported upstream and can help limit the spread of hybridization above barriers.

The methods we developed to assist with transport decisions for bull trout collected at Clark Fork River dams could have applications for bull trout and other native fish species in other watersheds as well. Many mainstem dams that fragment migratory corridors important to bull trout presently lack fish passage facilities, and habitat fragmentation is believed to be one of the primary factors limiting bull trout recovery range-wide (Fraley and Shepard 1989; Rieman et al. 1997; US Fish and Wildlife Service 2002). For example, prior to the removal of Milltown Dam on the upper Clark Fork River, Schmetterling (2003) collected bull trout below the dam and observed them in spawning areas following upstream transport. The Clark Fork River is an important migratory corridor for other native fish species as well. Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) are found in many LPO–CFR tributaries, and migratory adults have been collected below Cabinet Gorge and Noxon Rapids dams (Ardren et al. 2008). The effects of Milltown Dam have also been documented for native westslope cutthroat trout (Schmetterling 2003) and largescale suckers (*Catostomus macrocheilus*; Schmetterling and McFee 2006). Concerns associated with fragmentation of migratory corridors by mainstem dams exist in other watersheds as well. At Libby Dam on the Kootenai River in Montana, Dunnigan et al. (2009) documented entrainment of bull trout, and Paragamian et al. (1999) documented entrainment of native burbot (*Lota lota*).

Although our results demonstrate the utility of genetic assignment data for making fish transport decisions, additional biological factors may be equally important. These may include previous fish capture and movement information and habitat availability in the most likely population or region of assignment. For example, when the confidence in genetic assignments is low, it may be beneficial to compare the available habitat types in each assigned population or region and decide if a fish would benefit more being transported to one tributary or region versus the other. Other species may have limited or no genetic differentiation between populations or regions upstream or downstream of migration barriers, in which case population abundance may be the primary factor influencing transport decisions. Furthermore, populations that are genetically distinct may be ecologically interchangeable (Crandall et al. 2000), and ecological data such as the presence of non-native species and available habitat above and below barriers should also be considered carefully when developing fish transport programs. Although the biological and ecological variables will change depending on the watershed, additional information should be considered along with genetic data during the decision making process.

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Appendix A

Tables A1 and A2 appear on following pages.

Table A1. Fluvial distances in river kilometres between river mouths (above the diagonal) and pairwise estimates of genetic

Tributary No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	—	9.8	55.7	89.4	82.0	132.6	128.4	145.8	135.8	110.8	140.8	126.5	132.5	129.9	88.3	140.8	110.5
2	0.045	—	65.5	99.2	91.8	142.4	138.2	155.6	145.6	120.6	150.6	136.3	142.3	139.7	98.1	150.6	120.3
3	0.172	0.207	—	33.7	26.3	188.3	184.1	201.5	191.5	166.5	196.5	182.2	188.2	185.6	144.0	196.5	166.2
4	0.158	0.200	0.199	—	7.4	222.0	217.8	235.2	225.2	200.2	230.2	215.9	221.9	219.3	177.7	230.2	199.9
5	0.104	0.134	0.156	0.079	—	214.6	210.4	227.8	217.8	192.8	222.8	208.5	214.5	211.9	170.3	222.8	192.5
6	0.131	0.197	0.250	0.250	0.180	—	4.2	57.4	47.1	72.4	16.6	6.1	7.8	2.7	44.3	16.6	22.1
7	0.115	0.176	0.230	0.230	0.172	0.040	—	53.2	42.9	68.2	12.4	1.9	3.6	1.5	40.1	12.4	17.9
8	0.087	0.149	0.206	0.212	0.152	0.070	0.048	—	10.3	85.9	65.6	51.3	56.8	54.7	57.8	65.6	35.3
9	0.112	0.172	0.236	0.237	0.162	0.095	0.066	0.051	—	75.6	55.3	41.0	46.5	44.4	47.5	55.3	25.0
10	0.144	0.210	0.269	0.270	0.195	0.082	0.077	0.082	0.111	—	80.6	66.3	71.8	69.7	28.1	80.6	50.3
11	0.112	0.168	0.253	0.257	0.183	0.063	0.036	0.039	0.057	0.089	—	14.3	8.8	13.9	52.5	0.0	30.3
12	0.132	0.200	0.266	0.270	0.197	0.071	0.037	0.066	0.085	0.111	0.051	—	5.5	3.4	38.2	14.3	16.0
13	0.113	0.182	0.252	0.251	0.177	0.086	0.057	0.048	0.081	0.110	0.024	0.064	—	5.1	43.7	8.8	21.5
14	0.121	0.189	0.240	0.248	0.178	0.044	0.022	0.044	0.075	0.087	0.030	0.062	0.054	—	41.6	13.9	19.4
15	0.126	0.193	0.251	0.257	0.202	0.101	0.078	0.054	0.079	0.106	0.055	0.084	0.083	0.090	—	52.5	22.2
16	0.104	0.165	0.214	0.220	0.158	0.068	0.045	0.030	0.065	0.073	0.026	0.063	0.044	0.043	0.056	—	30.3
17	0.142	0.213	0.282	0.279	0.209	0.117	0.099	0.070	0.090	0.112	0.076	0.100	0.107	0.107	0.057	0.079	—
18	0.118	0.181	0.248	0.241	0.183	0.100	0.083	0.058	0.079	0.118	0.068	0.090	0.100	0.078	0.087	0.060	0.089
19	0.103	0.157	0.219	0.216	0.154	0.095	0.082	0.064	0.073	0.095	0.062	0.097	0.090	0.085	0.075	0.063	0.065
20	0.097	0.157	0.197	0.196	0.143	0.095	0.078	0.060	0.075	0.109	0.069	0.088	0.084	0.081	0.100	0.067	0.090
21	0.157	0.213	0.260	0.259	0.204	0.153	0.114	0.114	0.121	0.157	0.133	0.134	0.133	0.122	0.157	0.116	0.168
22	0.128	0.202	0.244	0.259	0.197	0.157	0.123	0.091	0.117	0.149	0.112	0.143	0.126	0.128	0.142	0.117	0.149
23	0.082	0.134	0.172	0.187	0.134	0.077	0.076	0.050	0.076	0.090	0.066	0.091	0.082	0.075	0.090	0.064	0.087
24	0.097	0.160	0.179	0.179	0.133	0.097	0.082	0.065	0.083	0.125	0.086	0.109	0.101	0.082	0.110	0.077	0.115
25	0.144	0.206	0.254	0.280	0.225	0.146	0.130	0.095	0.135	0.134	0.122	0.163	0.132	0.132	0.124	0.112	0.145
26	0.157	0.220	0.284	0.248	0.208	0.185	0.173	0.135	0.175	0.202	0.163	0.195	0.171	0.172	0.193	0.155	0.197
27	0.107	0.172	0.236	0.214	0.166	0.120	0.101	0.071	0.103	0.119	0.086	0.114	0.111	0.107	0.094	0.086	0.090
28	0.147	0.199	0.253	0.257	0.193	0.153	0.127	0.114	0.131	0.168	0.143	0.154	0.153	0.123	0.146	0.103	0.173
29	0.224	0.281	0.385	0.371	0.308	0.259	0.205	0.182	0.206	0.254	0.230	0.247	0.236	0.243	0.199	0.198	0.228
30	0.098	0.157	0.190	0.180	0.136	0.114	0.095	0.073	0.106	0.136	0.092	0.116	0.104	0.090	0.132	0.086	0.138
31	0.138	0.193	0.239	0.235	0.183	0.157	0.124	0.116	0.118	0.176	0.131	0.157	0.150	0.151	0.143	0.123	0.167
32	0.164	0.222	0.298	0.277	0.213	0.160	0.128	0.139	0.133	0.175	0.149	0.151	0.168	0.146	0.154	0.130	0.177
33	0.166	0.234	0.285	0.268	0.205	0.178	0.146	0.132	0.128	0.182	0.153	0.181	0.166	0.146	0.183	0.139	0.196
34	0.147	0.204	0.248	0.247	0.184	0.149	0.125	0.117	0.124	0.168	0.131	0.155	0.144	0.127	0.152	0.118	0.173
35	0.120	0.190	0.225	0.238	0.174	0.128	0.114	0.085	0.117	0.150	0.105	0.144	0.125	0.105	0.137	0.104	0.140
36	0.137	0.193	0.290	0.269	0.201	0.168	0.149	0.118	0.147	0.181	0.137	0.181	0.146	0.142	0.157	0.131	0.163
37	0.160	0.219	0.297	0.278	0.220	0.209	0.175	0.155	0.182	0.235	0.183	0.208	0.194	0.187	0.190	0.169	0.198
38	0.158	0.212	0.278	0.268	0.212	0.195	0.166	0.147	0.175	0.226	0.173	0.187	0.179	0.181	0.191	0.163	0.199
39	0.102	0.151	0.214	0.200	0.142	0.106	0.099	0.072	0.096	0.141	0.091	0.120	0.110	0.092	0.116	0.089	0.124

Note: Tributary numbers correspond to numbers in Table 1.

variation (F_{ST}; below the diagonal) among 39 bull trout spawning tributaries in the Lake Pend Oreille – Clark Fork River system.

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
121.7	162.4	156.7	163.7	205.5	217.9	242.4	182.9	228.5	253.3	345.2	429.2	375.1	322.2	656.4	541.7	555.4	459.7	623.5	366.3	372.3	496.7
131.5	172.2	166.5	173.5	215.3	227.7	252.2	192.7	238.3	263.1	355.0	439.0	384.9	332.0	666.2	551.5	565.2	469.5	633.3	376.1	382.1	506.5
177.4	218.1	212.4	219.4	261.2	273.6	298.1	238.6	284.2	309.0	400.9	484.9	430.8	377.9	712.1	597.4	611.1	515.4	679.2	422.0	428.0	552.4
211.1	251.8	246.1	253.1	294.9	307.3	331.8	272.3	317.9	342.7	434.6	518.6	464.5	411.6	745.8	631.1	644.8	549.1	712.9	455.7	461.7	586.1
203.7	244.4	238.7	245.7	287.5	299.9	324.4	264.9	310.5	335.3	427.2	511.2	457.1	404.2	738.4	623.7	637.4	541.7	705.5	448.3	454.3	578.7
25.5	66.2	60.5	67.5	109.3	121.7	146.2	86.7	132.3	157.1	249.0	333.0	278.9	226.0	560.2	445.5	459.2	363.5	527.3	270.1	276.1	400.5
21.3	62.0	56.3	63.3	105.1	117.5	142.0	82.5	128.1	152.9	244.8	328.8	274.7	221.8	556.0	441.3	455.0	359.3	523.1	265.9	271.9	396.3
46.5	87.2	81.5	88.5	130.3	142.7	167.2	107.7	153.3	178.1	270.0	354.0	299.9	247.0	581.2	466.5	480.2	384.5	548.3	291.1	297.1	421.5
36.2	76.9	71.2	78.2	120.0	132.4	156.9	97.4	143.0	167.8	259.7	343.7	289.6	236.7	570.9	456.2	469.9	374.2	538.0	280.8	286.8	411.2
61.5	102.2	96.5	103.5	145.3	157.7	182.2	122.7	168.3	193.1	285.0	369.0	314.9	262.0	596.2	481.5	495.2	399.5	563.3	306.1	312.1	436.5
33.7	74.4	68.7	75.7	117.5	129.9	154.4	94.9	140.5	165.3	257.2	341.2	287.1	234.2	568.4	453.7	467.4	371.7	535.5	278.3	284.3	408.7
19.4	60.1	54.4	61.4	103.2	115.6	140.1	80.6	126.2	151.0	242.9	326.9	272.8	219.9	554.1	439.4	453.1	357.4	521.2	264.0	270.0	394.4
24.9	65.6	59.9	66.9	108.7	121.1	145.6	86.1	131.7	156.5	248.4	332.4	278.3	225.4	559.6	444.9	458.6	362.9	526.7	269.5	275.5	399.9
22.8	63.5	57.8	64.8	106.6	119.0	143.5	84.0	129.6	154.4	246.3	330.3	276.2	223.3	557.5	442.8	456.5	360.8	524.6	267.4	273.4	397.8
33.4	74.1	68.4	75.4	117.2	129.6	154.1	94.6	140.2	165.0	256.9	340.9	286.8	233.9	568.1	453.4	467.1	371.4	535.2	278.0	284.0	408.4
33.7	74.4	68.7	75.7	117.5	129.9	154.4	94.9	140.5	165.3	257.2	341.2	287.1	234.2	568.4	453.7	467.4	371.7	535.5	278.3	284.3	408.7
11.2	51.9	46.2	53.2	95.0	107.4	131.9	72.4	118.0	142.8	234.7	318.7	264.6	211.7	545.9	431.2	444.9	349.2	513.0	255.8	261.8	386.2
—	40.7	35.0	42.0	83.8	96.2	120.7	61.2	106.8	131.6	223.5	307.5	253.4	200.5	534.7	420.0	433.7	338.0	501.8	244.6	250.6	375.0
0.064	—	25.3	32.3	74.1	86.5	111.0	51.5	96.8	121.6	213.8	297.8	243.7	190.8	525.0	410.3	424.0	328.3	492.1	234.9	240.9	365.3
0.072	0.053	—	7.0	48.8	61.2	85.7	26.2	71.8	96.6	188.5	272.5	218.4	165.5	499.7	385.0	398.7	303.0	466.8	209.6	215.6	340.0
0.123	0.133	0.088	—	41.8	54.2	78.7	19.2	64.8	89.6	181.5	265.5	211.4	158.5	492.7	378.0	391.7	296.0	459.8	202.6	208.6	333.0
0.118	0.105	0.081	0.158	—	12.4	36.9	22.6	23.0	47.8	139.7	223.7	169.6	116.7	450.9	336.2	349.9	254.2	418.0	160.8	166.8	291.2
0.072	0.064	0.048	0.110	0.091	—	24.5	35.0	10.6	35.4	127.3	211.3	157.2	104.3	438.5	323.8	337.5	241.8	405.6	148.4	154.4	278.8
0.093	0.073	0.053	0.128	0.107	0.031	—	59.5	35.1	59.9	151.8	235.8	181.7	128.8	463.0	348.3	362.0	266.3	430.1	172.9	178.9	303.3
0.121	0.115	0.102	0.154	0.158	0.071	0.105	—	45.6	70.4	162.3	246.3	192.2	139.3	473.5	358.8	372.5	276.8	440.6	183.4	189.4	313.8
0.151	0.133	0.118	0.201	0.160	0.126	0.125	0.190	—	24.8	116.7	200.7	146.6	93.7	427.9	313.2	326.9	231.2	395.0	137.8	143.8	268.2
0.087	0.052	0.070	0.158	0.093	0.069	0.080	0.131	0.152	—	141.5	225.5	171.4	118.5	452.7	338.0	351.7	256.0	419.8	162.6	168.6	293.0
0.147	0.114	0.128	0.186	0.195	0.114	0.112	0.192	0.197	0.135	—	84.0	29.9	28.4	311.2	196.5	210.2	114.5	278.3	154.9	160.9	151.5
0.226	0.196	0.205	0.254	0.234	0.195	0.236	0.283	0.283	0.199	0.258	—	54.1	112.4	227.2	112.5	126.2	30.5	194.3	238.9	244.9	67.5
0.085	0.084	0.056	0.111	0.091	0.055	0.064	0.116	0.115	0.097	0.121	0.226	—	58.3	281.3	166.6	180.3	84.6	248.4	184.8	190.8	121.6
0.133	0.108	0.093	0.148	0.125	0.099	0.097	0.157	0.175	0.122	0.145	0.230	0.097	—	339.6	224.9	238.6	142.9	306.7	131.9	37.9	179.9
0.130	0.112	0.114	0.168	0.174	0.121	0.124	0.186	0.215	0.119	0.141	0.202	0.134	0.143	—	114.7	101.0	196.7	380.9	466.1	472.1	213.3
0.133	0.129	0.103	0.155	0.138	0.113	0.114	0.153	0.183	0.141	0.182	0.271	0.090	0.145	0.114	—	13.7	82.0	272.2	351.4	357.4	98.6
0.127	0.115	0.094	0.152	0.156	0.094	0.089	0.163	0.175	0.130	0.141	0.232	0.103	0.134	0.093	0.080	—	95.7	285.9	365.1	371.1	112.3
0.101	0.088	0.083	0.150	0.106	0.069	0.094	0.123	0.149	0.105	0.139	0.254	0.059	0.130	0.160	0.104	0.123	—	190.2	269.4	275.4	37.0
0.130	0.100	0.102	0.184	0.154	0.103	0.120	0.150	0.175	0.140	0.167	0.287	0.093	0.172	0.194	0.176	0.165	0.107	—	433.2	439.2	227.2
0.176	0.166	0.153	0.213	0.181	0.152	0.162	0.215	0.209	0.170	0.223	0.291	0.158	0.192	0.208	0.199	0.202	0.187	0.218	—	6.0	306.4
0.171	0.168	0.153	0.213	0.183	0.155	0.170	0.223	0.212	0.164	0.210	0.268	0.168	0.198	0.201	0.212	0.195	0.180	0.217	0.073	—	312.4
0.079	0.069	0.068	0.143	0.116	0.068	0.073	0.136	0.120	0.075	0.108	0.239	0.073	0.107	0.134	0.137	0.116	0.089	0.131	0.166	0.163	—

Table A2. Proportion of individuals assigned to each tributary–genetic group identified using BAPS during leave-one-out

	Assigned to:													
Collected from:	Upper Priest River	Gold Creek	Indian Creek	MF East River	Ulnda Creek	Char–EF Lightning–Morris–Savage	Gold Creek	Granite Creek	Grouse Creek	Lightning–Porcupine–Rattle	Trestle Creek	Johnson Creek	Twin Creek	EF Bull River
Upper Priest River	**0.918**	0.041	0.041											
Gold Creek (Priest River)	0.000	**0.909**	0.023											
Indian Creek			**1.000**											
MF East River	0.032			**0.791**	0.174									
Ulnda Creek				0.313	**0.687**									
Char–EF Lightning–Morris–Savage						**0.883**	0.021		0.017	0.067	0.004		0.004	
Gold Creek						0.027	**0.767**	0.055		0.041	0.055	0.027		
Granite Creek							0.021	**0.965**						
Grouse Creek						0.014			**0.929**	0.043	0.014			
Lightning–Porcupine–Rattle						0.043	0.043		0.014	**0.870**			0.057	
Trestle Creek						0.020	0.032	0.020	0.000	0.020	**0.870**		0.013	
Johnson Creek							0.021					**0.957**	0.021	
Twin Creek						0.017							**0.797**	
EF Bull River														**0.821**
Rock Creek							0.013							
Swamp Creek														
Graves Creek														
Prospect–Cooper			0.095											0.005
Vermilion River														
Thompson River														
Fishtrap Creek														
Cedar Creek														
Fish Creek												0.018		
Little Joe Creek														
Copper Creek														
Montana Creek														
NF Blackfoot River														
Rattlesnake Creek														
Meadow Creek														
NF Jocko River														
SF Jocko River														
Upper Rock Creek														
Albert Creek														

Note: Values in bold represent the proportion of individuals assigned to the tributary they were collected from. MF, Middle Fork;

assignment tests.

Assigned to:																		
Rock Creek	Swamp Creek	Graves Creek	Prospect–Cooper	Vermilion River	Thompson River	Fishtrap Creek	Cedar Creek	Fish Creek	Little Joe Creek	Copper Creek	Montana Creek	NF Blackfoot River	Rattlesnake Creek	Meadow Creek	NF Jocko River	SF Jocko River	Upper Rock Creek	Albert Creek
							0.012										0.012	
																	0.004	
				0.027														
								0.011										
		0.007													0.007	0.007		
				0.006			0.006											
0.017		0.017		0.034			0.068	0.017									0.034	
0.021	0.011	0.032			0.011	0.042	0.011	0.011			0.011	0.011					0.021	
0.913		0.013				0.013					0.025						0.025	
	0.971																	
		0.970				0.030												
0.005	0.005	0.052	**0.780**	0.005	0.010	0.047	0.047	0.010		0.005			0.010				0.005	
				0.976		0.024												
0.014					**0.986**													
					0.012	**0.976**					0.012							
							1.060											
			0.018					**0.875**	0.018		0.018						0.036	
									1.005									
										1.000								
											0.946	0.054						
												1.000						
													1.000					
														1.000				
															0.970	0.030		
															0.032	**0.968**		
																	1.000	
																		1.000

EF, East Fork; NF, North Fork; SF, South Fork.

Fishing power increases from technological development in the Faroe Islands longline fishery

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Abstract: During an effort-regulated period from 1996 to 2002, unregistered annual increases of 0.3% of hooks fished per day were demonstrated for the Faroe Islands longline fishery. However, annual increases were higher (1.5%) during a preceding total allowable catch regulated period, thereby invalidating an expectation of the 1996 shift in regulations (output to input control) to have induced increases in the number of hooks set per day. Underlying this result is a substantial increase in total yearly effort (fishing days) and a shift in targeting behaviour from secondary to primary (high value) target species in response to the transition from output to input control. Interview data on technology were combined with logbook data and analysed with generalized linear modelling to demonstrate haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) catch-per-unit-effort increases of 51% and 26%, respectively, following the introduction of skewed hooks and swivel line. The technological introductions were not correlated to regulation shifts. So, rather than the management system in force, an ongoing technological development seems to be the principal driver of fishing power trends. The results highlight the need to explicitly address technological development and targeting behaviour when attempting to meet conservation objectives through input control of fisheries.

Résumé : De 1996 à 2002 durant une période de contrôle des efforts dans la pêche à la palangre des îles Féroé, nous avons observé des augmentations annuelles non enregistrées de 0,03 % des hameçons utilisés par jour. Cependant, les augmentations annuelles avaient été plus élevées (1,5 %) durant une période précédente de contrôle des captures totales permises, ce qui invalide la prédiction que le changement des règlements en 1976 (contrôle des entrées plutôt que des sorties) augmenterait le nombre d'hameçons utilisés par jour. Sous-tendant ce résultat, il y a un augmentation considérable de l'effort annuel total (jours de pêche) et une modification du comportement de ciblage de la pêche, des espèces secondaires aux espèces primaires (de grande valeur), en réaction à la transition du contrôle des sorties à celui des entrées. Nous avons combiné des données tirées d'entrevues sur la technologie à des données provenant des livres de bord et les avons analysées par modélisation linéaire généralisée pour démontrer des augmentations respectives de 51 % et de 26 % des captures par unité d'effort pour l'aiglefin (*Melanogrammus aeglefinus*) et la morue franche (*Gadus morhua*) après l'introduction d'hameçons obliques (skewed hooks) et de lignes à émerillons. Il n'y a pas de corrélation entre l'introduction des nouvelles technologies et les changements de règlementation. Ainsi, l'évolution continuelle de la technologie, plutôt que le système de gestion en place, semble être le principal facteur explicatif des tendances dans la puissance de la pêche. Ces résultats soulignent la nécessité d'étudier de façon explicite les développements technologiques et les comportements de pêche, lorsqu'on cherche à atteindre des objectifs de conservation de la pêche par un contrôle des entrées.

[Traduit par la Rédaction]

Introduction

Technological development in commercial fisheries often complicates efforts to balance capacity and resources by changing vessel fishing power (e.g., Standal 2005; Branch et al. 2006). The actual technologies and mechanisms influencing vessel fishing power are numerous and depend on both the catch process and the management regime in question. Previous studies have reported changes in vessel fishing power caused by the introduction of global positioning systems (GPS; Robins et al. 1998), twin trawl technology (Mahevas et al. 2004), and engine power development (Rijnsdorp et al. 2006; Eigaard and Munch-Petersen 2010). The effectiveness of various management systems, including capacity reduction plans (Pascoe et al. 2001), catch quota restrictions (Daan 1997), and technical regulations (Rijnsdorp et al.

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2008), has been adversely altered by technological developments. Other processes than technological development have been shown to induce unforeseen changes in catch efficiency, thereby hampering the achievement of management objectives. These include (*i*) fishermen behaviour (e.g., changed fishing practices in response to regulations, price conditions, or resource availability; Squires and Kirkley 1999; Marchal et al. 2006), (*ii*) competition for space between fishing vessels (Rijnsdorp et al. 2000), (*iii*) technical interactions (Ulrich, et al. 2001), and (*iv*) biological factors such as stock contractions and changed availability of target species due to environmental conditions (Rose and Kulka 1999; Steingrund et al. 2009). The main subject of this paper is the understanding of technological development in fisheries, its influence on catch efficiency of fishing vessels, and its interactions with the major regulation types (input and output control), but in dealing with this subject, the processes listed above will also be addressed at different levels.

The case study investigated is the Faroese longline fishery for Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). In general, longline fishery is considered an environmentally friendly fishery with low fuel consumption, little bottom impact, and low catches of undersized fish (Løkkeborg and Björdal 1992; Hovgaard and Lassen 2000). Thanks to a series of technologically oriented skipper interviews and questionnaires, this fishery offers a unique opportunity to analyze fishermen behaviour and technologically driven trends in fishing power across a period with three different management systems in force. The longline fishery is currently regulated by an effort quota system (yearly days at sea) but was previously regulated by a set of technical measures (1986–1993) and then by a total allowable catch system (1994–1995). Because there are only few examples of large-scale fisheries regulated by pure effort quotas worldwide, important lessons can be learned from the Faroese management experiences. These can add to the knowledge base for modern fisheries management and contribute to the current exploration and discussion of input versus output based conservation instruments (e.g., Motos and Wilson 2006; Andersen et al. 2010; Baudron et al. 2010).

The obvious advantage of output control is a seemingly inevitable agreement between intended and realized fishing mortality; when the total allowable catch (TAC) of a species is reached, the fishing for this species is stopped. The main weakness of output control is its inability to cope with discards and misreported catches, which undermine the idea of controlling fishing mortality through control of fishermen landings and complicates the assessment of stock size (Raakjær Nielsen and Mathiesen 2003; Catchpole et al. 2005; Branch et al. 2006). As a consequence of discards and misreporting, stock assessment and thus the basis of setting TACs can be substantially biased (Daan 1997; Rijnsdorp et al. 2007). The setting of appropriate TAC levels can also be hampered by catchability changes owing to technological development (Salthaug 2001; Marchal et al. 2002).

The main advantages of short-term input control (e.g., a yearly effort quota of days at sea) are argued to be easy enforcement and monitoring; improved regulation legitimacy and compliance among fishermen; and limited incentives to discard, high-grade, or misreport (Rossiter and Stead 2003; Shepherd 2003). Consequently, the basis for scientific stock assessments (the officially reported catch and effort data) will be reliable, dumping and waste of caught fish will be minimized, and improved accordance between intended and realized fishing mortality will be obtained. The main shortcoming of input control as a fisheries management strategy is its vulnerability to technological development and the resulting efficiency changes of vessels (Stefansson and Rosenberg 2005; Jákupsstovu et al. 2007; Eigaard 2009). Technological development makes it inevitable to somehow meet efficiency increases with compensating reductions in allocated fishing days or any other chosen measures of effort with regular intervals (Cadwallader et al. 2000; Martell and Walters 2002; Catchpole et al. 2005). Another problematic aspect of input control in mixed fisheries is the ability of fishermen to target any species of fish they wish, including high-value species with low stock levels, which in theory could be fished to extinction (Rossiter and Stead 2003).

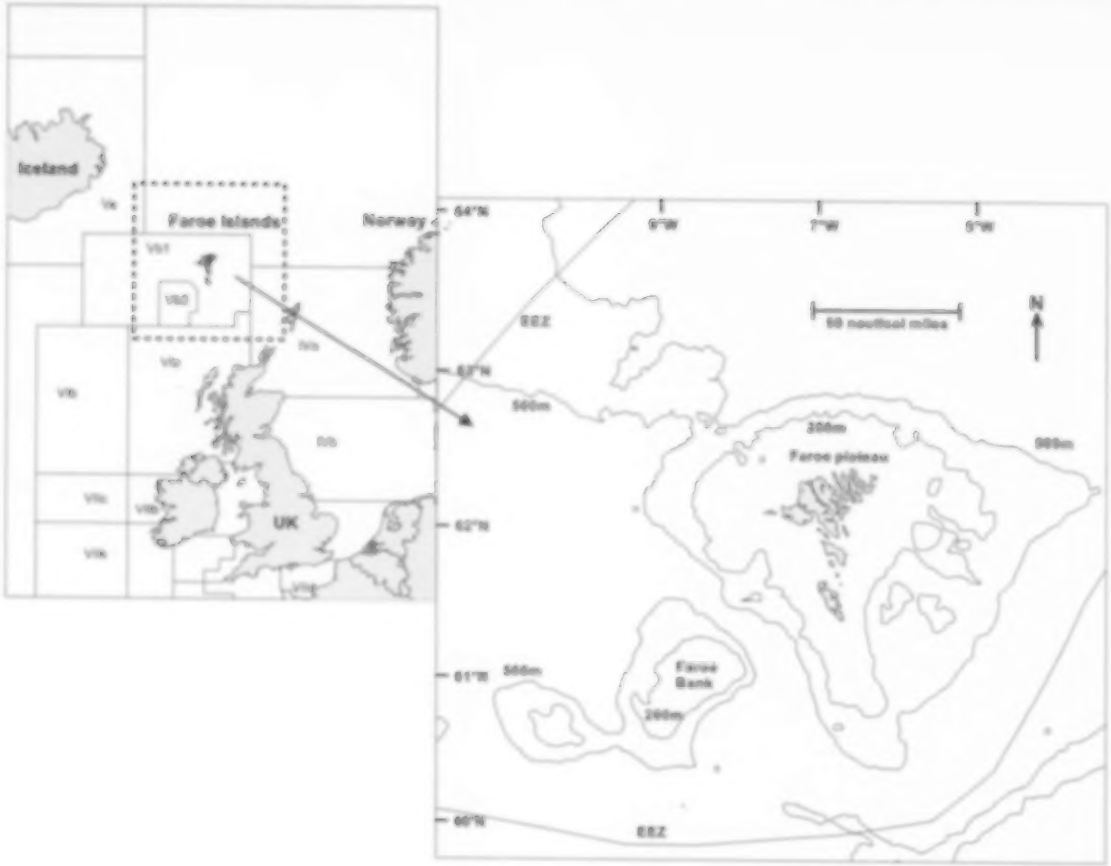
With the analyses and results presented in this paper, we aim to examine the effects of changed targeting behaviour and technological development on fishing power of the Faroese longliners across three different management regimes (a technical measures system (TM), a TAC system, and a total allowable effort system (TAE)) during a 17-year period from 1986 to 2002. Based on interview information from the longline skippers and existing literature on longline fishing power trends (e.g., Sainsbury 1996; Hovgaard and Lassen 2000), we hypothesize that substantial increases in fishing power have taken place in the Faroese longline fishery. This hypothesis is tested with generalized linear modelling (GLM) of a data set combining (*i*) interview data on technology uptake in the fleet from 1986 to 2002, (*ii*) logbook data on catch and effort for the same period, (*iii*) cod and haddock stock indices from fishery-independent surveys for the same period, and (*iv*) a primary production index covering the years 1990 to 2002. The longliner targeting behaviour is explored qualitatively using logbook data. The results and conclusions are first discussed in the light of the general perception that the effort-based management system on the Faroe Islands has failed to meet target fishing mortalities for the demersal fleet (e.g., Jákupsstovu et al. 2007; Baudron et al. 2010), before more general inferences with respect to the technological caveats of input- and output-oriented fisheries management are drawn.

Materials and methods

Fisheries management on the Faroe Islands

On 1 June 1996, the Faroe Islands introduced an effort-based management system. For all vessels targeting cod, haddock, and saithe (i.e., pollock, *Pollachius virens*), the effort system was based on individual transferable effort quotas (yearly fishing days) by vessel group (defined mainly by gear type and vessel size) and fishing area (defined mainly by distance to coast and water depth (<200 m or ≥200 m)). Prior to the introduction of the effort system, the Faroese fishery was regulated by a TAC regulation from 1 September 1994 to 31 May 1996 and before this by licence limits and a set of technical measures: area restrictions for trawl fisheries, minimum mesh sizes, measures to protect juvenile fish, and others. The TAC system was introduced as a consequence of cod, haddock, and saithe stock collapses in the early 1990s.

Fig. 1. The main fishing ground for the longliners, the Faroe Plateau ground, is the shallow water (less than approximately 200 m depth) around the Faroe Islands (1 n.mi. = 1.852 km).



However, some resistance from the industry led decision makers to replace the TAC system by direct effort regulation in 1996 (Jákupsstovu et al. 2007).

The Faroese cod and haddock stocks

The International Council for Exploration of the Sea (ICES) assesses two distinct cod stocks within area Vb (ICES 2008): the Faroe Plateau cod (Vb1) and the Faroe Bank cod (Vb2), separated by the deep waters (>500 m depth) of the Faroe Bank channel (Fig. 1).

The Faroe Plateau cod stock is the largest in terms of both stock size and landings, with an estimated spawning stock biomass (SSB) averaging close to 60 000 tonnes (t) during the latest five decades and annual landings averaging close to 25 000 t. Since 2003, however, the SSB has declined steeply, and all following years the advice from ICES has been no fishing and development of a rebuilding plan. Fishing has, however, continued.

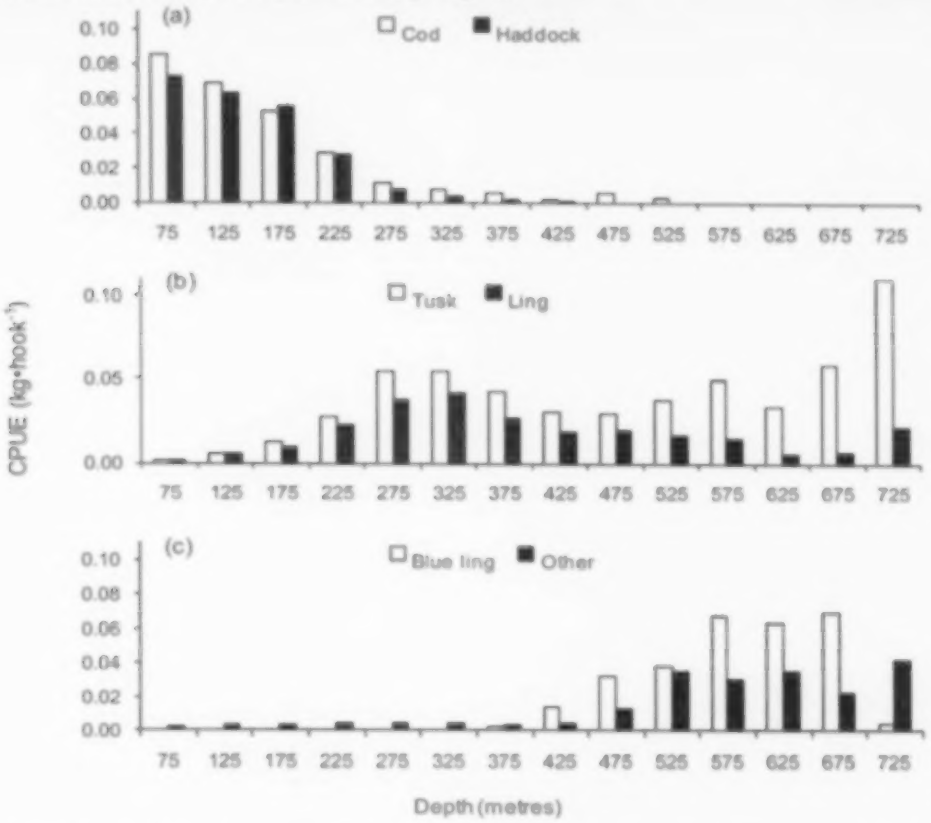
The Faroe Bank cod stock has no analytical assessment or biological reference points, but survey indices indicate that the stock is severely depleted. Landings have declined steeply in the last 3 years from 5500 t in 2003 to only 500 t in 2007, while exploitation ratios (proxy for fishing mortality) remain higher than average.

Only one haddock stock is defined within area Vb (Vb1 +Vb2), with an estimated SSB averaging ~60 000 t during the last five decades and annual landings averaging close to 15 000 t yearly. From a historical maximum of 100 000 t in 2003, the SSB has, however, declined steeply to only 42 000 t in 2008, slightly above the precautionary biomass (B_{pa}) of 35 000 t (ICES 2008).

The Faroese longline fishery

In 2007, the vessel group "large longliners" (LLL) consisted of 25 vessels larger than 110 gross register tons (1 register ton = 2.83 m^3). The target shares of total annual Faroese catches of cod and haddock assigned to the large longliners are 23% and 28%, respectively. The average annual landing value of cod and haddock of that vessel group is estimated by Jákupsstovu et al. (2007) to be roughly 13 and 8 million euros, respectively. Other economically important species for the large longliners are European ling (*Molva molva*), blue ling (*Molva dypterygia*), and tusk (*Brosme brosme*). Typically, catches of these secondary species become more dominant during the summer period, May to September, when the availability of cod and haddock is lower and the longlines are set at greater depths. Practically no other species than cod and haddock are caught at depths less

Fig. 2. The average CPUE by depth of (*a*) cod and haddock, (*b*) tusk and European ling, and (*c*) blue ling and "other" of the examined period (1986–2002), calculated from catch and effort data from five large longliners.



than 200 m, whereas secondary species become increasingly dominant with greater depths (Fig. 2). The typical trip duration is 5–6 days and usually 25 000 to 35 000 hooks are set per day.

Data material

The analyses and results presented are based on data from three sources. The first data source is a set of official catch and effort data for five large Faroese longline vessels covering the period 1986 to 2002. The five vessels, ranging from 30.7 to 38.6 m in length, represent approximately 25% of the total group of large longline vessels of the period (19 licenses in 2002). This data set holds 13 688 observations of variables such as catch mass of individual species (cod, haddock, European ling, blue ling, and tusk), total catch mass, fishing depth, fishing area (ICES rectangle), and the number of hooks set for each day fished in the 17-year period.

The second data source is the information from an interview program conducted by the Faroese Fisheries Laboratory in 1997 and updated in 2003 to fill in gaps of information. This program aimed at retrospectively identifying technology acquired by vessels of the LLL group, and in particular technologies with a marked effect on the catch efficiency in the longline fishery. The reports from interviews with the skippers of the five longline vessels described above were kindly made available for this analysis by the Faroese Fisheries Laboratory. The five vessels were picked for the interviews based on the reliability and consistency of logbook records (data source 1). The identified technologies, time span of introduction, and estimated effect on catchability, together with changes in gear deployment or fishing practices expected to affect the reliability of fishing days as a descriptor of effective effort, are shown (Table 1).

The third data source is the information from a questionnaire survey conducted by the Faroese Fisheries Laboratory in 2000 addressing changes in catch efficiency of the entire Faroese fleet. More than 200 skippers from a variety of vessel types such as trawlers, seiners, gillnetters, and longliners responded to the questionnaire. The answers give an overview of when technological changes have been made to vessels and gears as well as the related efficiency change as estimated by the individual skippers. Technological innovations, time span of introduction, and estimated catch improvements from skippers in the large longliner vessel group are listed below (Table 2).

Key technologies affecting fishing power

The skipper interviews pointed to the swivel line and the skewed hooks as the most important innovations of the surveyed period (Fig. 3). Four out of the five vessels estimated both innovations to have had a substantial positive effect on catchability (Table 1). This evaluation was strongly backed by

Table 1. Technological innovations, their prevalence, and estimated effect on catchability for the five picked vessels of the large longliners (LLL) group as informed in skippers' interviews in 1997.

Innovation	Introduction	Abundance	Positive catch effect	No catch effect	Estimate not given	Comment
Automatic baiter	1978–1986	100%	—	—	5	—
Plotter	1983–1989	100%	1	1	3	—
Skewed hooks	1991–1995	100%	4	—	1	Particularly haddock
Swivel line	1992–1995	100%	4		1	Particularly haddock
GPS	1992–1994	100%	2	2	1	—
Stability tanks	1995–2002	80%	2	2		—
Changes in lines set daily	1976–1994	100%	—	—	5	—
Changes in hooks per line	1976–1994	80%	—	1	3	—

Table 2. Technological innovations and their estimated effect on catch rates for vessels in the LLL group from a 2000 questionnaire survey covering the entire Faroese fleet.

Innovation	Year	Completed questionnaires*	Range of catch improvements	Mean catch improvement	Comment
Automatic baiter	1978–1993	18 (9)	0%–30%	16%	—
Skewed hooks	1990–1996	17 (14)	5%–50%	21%	—
Stability tanks	1991–1999	10 (5)	5%–30%	13%	—
Swivel line	1992–1996	17 (14)	10%–50%	34%	Particularly haddock
Snood line	1992–1996	5 (3)	10%–20%	13%	—
Others	1991	1 (0)	—	—	—

*The numbers in parentheses indicate the responders that gave an estimate of catch improvement.

the questionnaire survey, where 14 out of 17 vessels having acquired skewed hooks and swivel line estimated the catch improvements to be on average 21% and 34%, respectively (Table 2). Both sources of information indicated that haddock catch rates were influenced most by the two innovations.

Other widespread innovations identified from interviews and questionnaires were automatic baiters, GPS, plotters, and stability tanks. Of these technologies, only the GPS and the stability tank were broadly introduced during the study period (Fig. 4) and assessed to result in catch improvements (Table 1 and Table 2). Therefore, we have focused the following analyses on identifying the CPUE influence from the introduction of the swivel line, skewed hooks, GPS, and stability tanks in the Faroese longline fishery.

In addition to identifying the key technologies expected to influence catchability, the interviews also established that all five vessels had implemented changes in gear deployment, which might have influenced the reliability of fishing days as a descriptor of effective effort (Table 1). This had taken place by setting more — in some cases less — lines per day by changing the ganglion distance and thereby the number of hooks on the lines or by deploying longer or shorter lines. The motives behind the changes were not clearly stated in the interviews, and no estimates of catch influence were given. It was, however, clear from the interviews that effective effort (hooks fished per day) had varied substantially during the period prior to the interviews.

Trends in longline fishing effort and targeting behaviour

Total yearly number of hooks set by the five longliners on the Faroe Plateau was calculated and graphed and related to the shifting management regimes of the examined period. To evaluate whether the five investigated longline vessels had changed their cod- and haddock-directed effort share in response to the shifting management regimes of the period, the yearly number of hooks set at 200 m depth or more and at less than 200 m was calculated and graphed.

Modelling variations in hooks fished per day

The variation in average number of hooks fished per day of the five large longliners (data source 1) was analysed using a GLM (McCullagh and Nelder 1995). Only trips to the main fishing ground, Vb1, of the large Faroese longliners (Fig. 1), were included in the analysis. To exclude irregular fishing days, due to, for example, weather conditions or material break down, 76 fishing days with less than 10 000 hooks set were excluded from the data set (1.15% of the observations). To estimate the total year-to-year changes, the hook number fished per day (E) was modelled in response to three class variables, a year factor (Y), as well as a vessel (V) and a seasonal (S) factor:

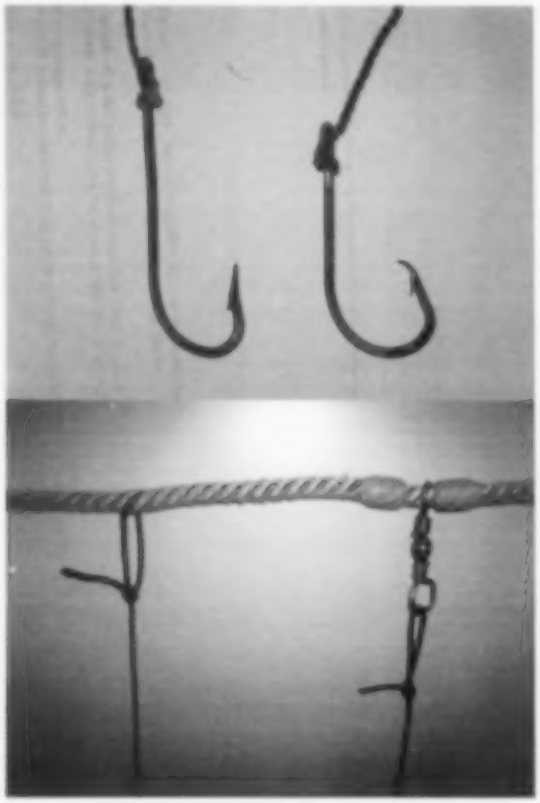
$$(1) \qquad E_{ijk} = \beta_0 + V_i + S_j + Y_k + \varepsilon_{ijk}$$

To estimate the year-to-year changes taking into account variation in the species targeted, the hook number fished per day (E) was modelled in response to a year × depth interaction ($Y \times D$) as well as a vessel (V) and a seasonal (S) factor:

$$(2) \qquad E_{ijkl} = \beta_0 + V_i + S_j + Y_k \times D_l + \varepsilon_{ijkl}$$

Model 1 and Model 2 were parameterized on the basis of 6609 days of fishing. The number of hooks fished per day (E) varied from 10 000 to 57 000, i denotes the ID number of the five vessels, j denotes the twelve months of a year, k denotes the years from 1986 to 2002, l denotes the depth fished (<200 m and ≥200 m), and ε is a random noise, assumed to be normally distributed. This assumption was validated by plotting the standardized residuals against the

Fig. 3. The key longline development of the study period: the transition from a straight to a skewed hook and from a fixed ganglion line attachment to a fully flexible swivel line attachment.



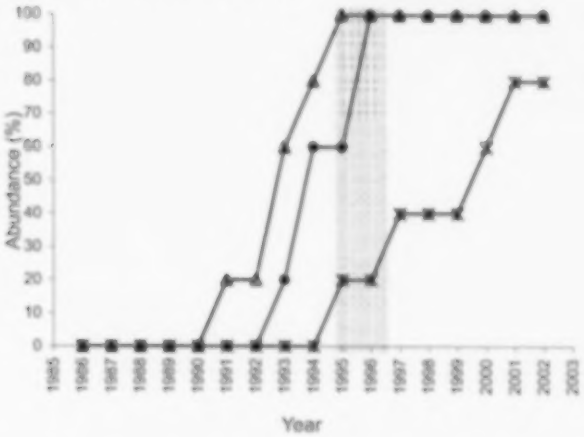
quantiles of standard normal distribution (QQ-plot). Homogeneity and independence was verified by plots of residuals versus fitted values and explanatory variables. Calculations were made using the GLM procedure of the SAS software (SAS Institute Inc. 2004). Multiplicative versions of the models were explored, but a comparison of QQ-plots indicated that an additive model was better suited to the error structure of the data (Fig. 5).

Modelling the influence of new technologies on vessel fishing power

To estimate the influence of the identified key technologies (swivel line, skewed hooks, GPS, and stability tanks) on cod and haddock catchability, the interview data on technological acquisitions of the five longline vessels (data source 2) were merged by date with their logbook data on catch and effort for the period 1986 to 2002 (data source 1). Observations before introduction were assigned a "no" and observations after introduction were assigned a "yes" for each new technology variable.

It proved difficult to separately estimate the catchability effect from swivel line and skewed hooks, as they were introduced almost simultaneously, and in some cases together, on the individual vessels. We therefore decided to treat swivel line (SL) and skewed hooks (SH) as one joint technological

Fig. 4. Temporal introduction pattern of the three investigated technologies: the swivel line and skewed hooks (SL–SH, triangles), the global positioning system (GPS, solid dots), and the stability tanks (ST, stars). The shaded area indicates the total allowable catch (TAC) period (1994 to 1996), separating the technical measures (TM) period (1986 to 1994) and the total allowable effort (TAE) period (1996 to 2002).



acquirement (SL–SH) when merging the interview information to the logbook information of catch and effort. Furthermore, swivel line and skewed hooks were not always purchased as single discrete events but gradually replaced existing lines and hooks during typically a 1- to 2-year period. Ideally, separate catch records of simultaneous use of SL–SH and non-SL–SH gears on board the vessels would be available. Unfortunately this was not the case, and observations from time periods where we were uncertain of the degree of SL–SH introduction were left out of the analyses.

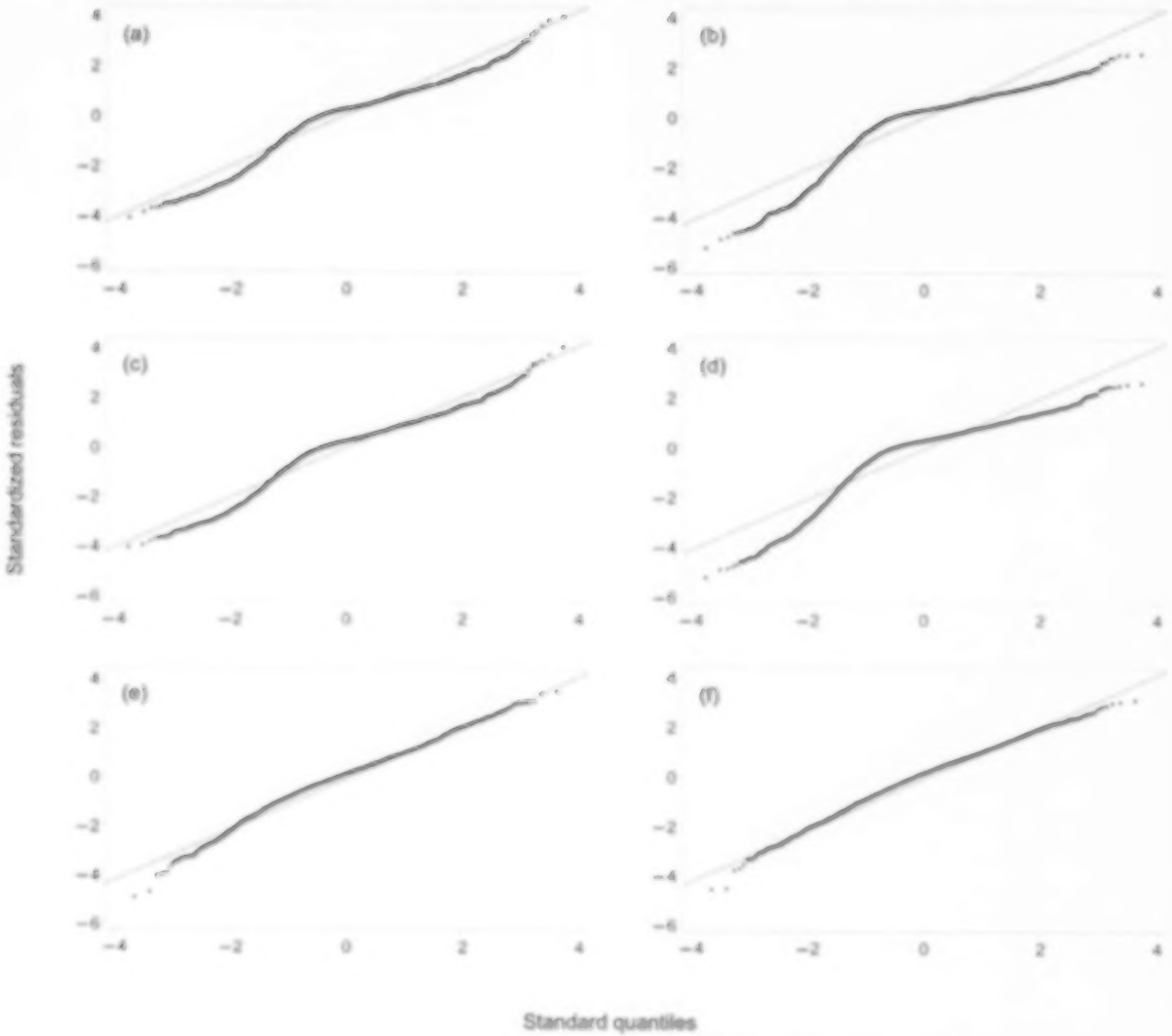
To ensure that cod and haddock were the main target species of the trips modelled, only observations with the lines set shallower than 200 m depth were included (Fig. 2). Furthermore, all catch observations from ICES squares outside the Faroe Plateau were excluded as were all observations without a specified hook number.

A fishery-independent survey index (SI) of stock size of cod and haddock on the Faroe Plateau was included in the GLM to eliminate yearly variation in abundance. The stock indices for the period 1986 to 2002 were based on trawl data from trawl surveys with the research vessel *Magnus Heinason* (ICES 2008).

Year-to-year variations in food abundance and catchability of cod and haddock to longlines was accounted for by including a primary production index (PP) for the Faroe Plateau (ICES 2008) in the GLM analysis. Negative correlation between high food abundance and longline catchability has been demonstrated for the Faroe Plateau cod (Steingrund et al. 2009), and the assumption is made that a similar correlation exists for haddock. The PP index was squared to accommodate an assumption of nonlinear correlation based on the existence of intermediate food chain levels between primary production and cod.

The combined data set was used for analysing variation in daily CPUE values (kg·hook^{-1}) in response to five factors: the presence (yes) or absence (no) of SL–SH, GPS, stability

Fig. 5. QQ-plots of standardized residuals from (*a*) model 1 — nominal number of hooks fished per day, (*b*) model 1 — log-transformed number of hooks fished per day, (*c*) model 2 — nominal number of hooks fished per day, (*d*) model 2 — log-transformed number of hooks fished per day, (*e*) model 3 — log-transformed cod CPUE values, and (*f*) model 3 — log-transformed haddock CPUE values.



tanks (ST), an area factor (*A*), and a seasonal factor (*S*), as well as two continuous indices: PP and SI, with multiplicative modelling (Gulland 1956; Robson 1966; Gavaris 1980). A vessel factor was left out of the model to reduce the risk of confounding with the three vessel-based technology factors (Maunder and Punt 2004). A traditional GLM approach was considered appropriate; zero CPUE values are few (2.2% of the observations for cod and 3.3% for haddock), and the assumption of log-normally distributed CPUE values cannot be rejected based on plots of standardized residuals against the quantiles of standard normal distribution (QQ-plots) for cod and haddock (Fig. 5).

Industry information pointed to the vessels being able, to some extent, to target either cod or haddock in the individual sets through choice of bait and location. To take into account possible CPUE bias from sets where the "other" species was the main target, or where the lines were unintendedly saturated with other species (Hovgaard and Lassen 2000), cod and haddock CPUEs were treated separately in two models and catch mass of the other species was included in the models as a continuous variable (*O*).

All variables described above were tested using the log-likelihood value of the full parameterized model as a baseline. Model reductions (the GPS variable) were made at a 0.01% significance level (log-likelihood ratio test), resulting in the following generic end model for cod and haddock:

$$(3)\quad \log(\mathrm{CPUE}_{ijkl}) = \beta_0 + \mathrm{SL} - \mathrm{SH}_i + \mathrm{ST}_j + A_k + S_l + \mathrm{PP}^2 + \mathrm{SI} + O + \varepsilon_{ijkl}$$

Model 3 was parameterized on the basis of 4409 and 4315 daily catch records of cod and haddock, respectively. The CPUE values ranged from 0 to 0.529 kg·hook^{-1} (cod) and 0 to 0.371 kg·hook^{-1} (haddock). β_0 is the common fixed intercept, i and j denote the two categories "yes" and "no", k denotes 17 different ICES rectangles of the Faroe Plateau, l denotes the 12 months of a year, PP denotes primary production index value of the plateau ranging from 1 to 5.5, SI denotes stock index value ranging from 1 to 17.1 (cod) and 1 to 14.3 (haddock), O denotes the catch mass of the other species ranging from 0 to 16 t (cod) and 0 to 13 t (haddock), and ε is a random noise, assumed to be normally distributed (an assumption validated by QQ-plots). Calculations were made using the GLM procedure of the SAS software (SAS Institute Inc. 2004).

Results

Trends in longline fishing effort and targeting behaviour

The total yearly number of hooks set by the five large longliners almost doubled following the shift from the TAC to the TAE system (Fig. 6*a*). When graphed by depth, this increase in yearly effort can be observed to have taken place only in the cod- and haddock-directed fisheries (hooks set at less than 200 m depth), whereas the hook number set for secondary target species such as tusk, European ling, and blue ling (hooks set at 200 m or more) has actually declined (Fig. 6*b*). Consequently, a marked shift in targeting behaviour, from secondary to primary (high value) target species, has taken place in response to the transition from output to input control. Figure 6*b* also gives some indication that the opposite shift (from primary to secondary target species) was induced by the previous shift from the TM system to the TAC system (from an input- to an output-oriented regulation) in 1994. The signal is, however, somewhat blurred because the regime shifts have taken place intra-annually, which is poorly captured in the plots by year.

Regression analysis of hooks fished per day

There were significant differences ($P < 0.0001$) among 6 of the 17 model 1 year estimates, and in the period from 1986 to 1991 less hooks were fished per day compared with 2002. A linear fit to the individual year estimates of total number of hooks set per day (Fig. 6*c*) resulted in a 1.3% annual average increase ($R^2 = 0.85$). This annual increase appears to occur more or less gradually across the entire study period, and no obvious differences exist among the three different management regimes. Possibly there is a flattening of the index curve towards the end of the TAE period, and for this period as a whole there is an average annual increase of 0.3%. For the TAC period the increase is 1.5%. Observing that there are also periods of zero or negative growth during the TM period (1.6% increase across entire period), it is very doubtful whether the rates of increase in total number of hooks set per day can be said to differ significantly among the three management regimes. When the number of hooks fished per day is plotted by depth (model 2 year estimates), it is observed that the shift from output to input control in 1996 induced a drop in the number of hooks set daily for the secondary target species (hooks set at 200 m or more), but towards the end of the period this drop has been set off again by a gradual increase (Fig. 6*d*). The somewhat different trends in hooks per year by depth (Fig. 6*b*) and hooks per day by depth (Fig. 6*d*) reflect that rather than setting more hooks per day following the transition from output to input control, the vessels have increased their annual fishing days.

Both model 1 and model 2 estimated one vessel, vessel #3, to set a significantly ($P < 0.0001$) higher (5.0% and 5.3%, respectively) number of hooks per day compared with the others vessels. Both models also estimated one vessel, vessel #1, to set a significantly lower (4.1% and 3.1%, respectively) number of hooks per day, but in general the vessel group appears homogeneous with respect to the number of hooks fished per day. None of the monthly estimates were significantly different ($P < 0.0001$) in any of the models.

Regression analysis of technological influence on fishing power

Haddock CPUE increased significantly ($P < 0.0001$) with a factor 1.51 following the introduction of swivel line and skewed hooks, and with a factor 1.34 following the introduction of stability tanks. Cod CPUE was somewhat less affected by the two technologies, a factor 1.26 and 1.09, respectively (Table 3). Neither CPUE was significantly influenced by the introduction of GPS on board the vessels. In both models, catch mass of the "other" species had a significant negative influence on CPUE. Cod CPUE decreased with a factor 0.96 with each 1 t increase in haddock catch of a trip, and haddock CPUE decreased with a factor 0.92 with each 1 t increase in cod catch (Table 3). The PP index had significantly negative effect on both haddock and cod CPUE with a 1% decrease in CPUE with each increase in the squared index value of 1. CPUE of both species was significantly influenced by the stock indices (a factor 1.10 for cod and a factor 1.08 for haddock with each increase in index value of 1; Table 3). For cod, 10 of 17 area effects (ICES rectangles) differed significantly (a factor 0.24 to a factor 1.83). For haddock, four area effects were significantly different (0.68 to 1.37). The seasonal effects (months) were indexed relative to December and graphed (Fig. 7). Moderate seasonal differences in cod and haddock CPUE values exist, with slightly higher haddock CPUE values in the early spring and autumn and slightly lower haddock CPUE values in the early summer.

Discussion

The state of the Faroese cod and haddock stocks has deteriorated during the last 25 years, and the present effort regulation system, introduced in 1996, has apparently not been able to change this development (Jákupsstovu et al. 2007; ICES 2008; Baudron et al. 2010). We reason that underestimating technical creep has adversely affected the conservation performance of the effort-based Faroese management system.

We hypothesized that owing to the poor quality of fishing days as descriptor of fishing effort, unregistered increases in fishing power (number of hooks fished per day) have taken place in the longline fishery. Modelling results confirm unregistered annual increases in hooks set per day of approximately 0.3% in the cod- and haddock-directed longline fishery during the TAE period, thus explaining some of this systems' failure to achieve agreement between intended and

Fig. 6. Estimates of (*a*) development in total yearly hook number, (*b*) total yearly hook number by depth (<200 m, circles; and ≥200 m, crosses), (*c*) model 1 year factors with standard errors and a linear fit, and (*d*) model 2 estimates of year factors by depth (<200 m, circles; and ≥200 m, crosses) with standard errors. The shaded area indicates the TAC-regulated period (1994 to 1996), separating the TM period (1986 to 1994) and the TAE period (1996 to 2002).

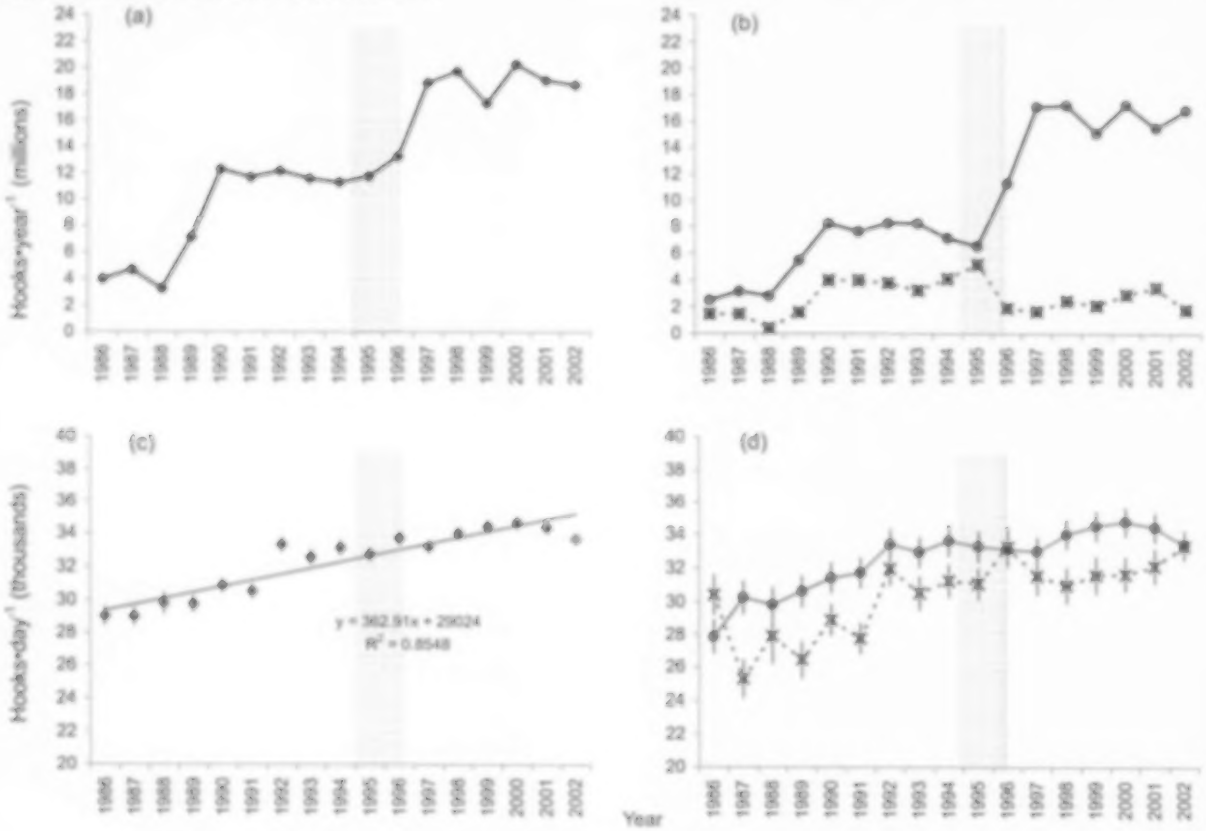


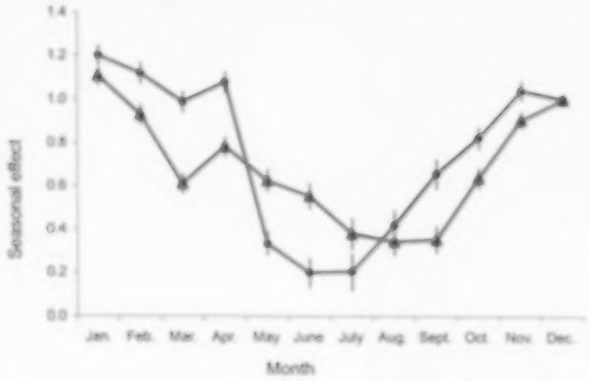
Table 3. Estimates of model 3a (cod) and 3b (haddock) parameters and statistical test values.

Species	df	Variable	Parameter estimate	Standard error	$P > \|t\|$
Cod	4408	Mean CPUE	0.024	0.001	<0.0001
		Swivel line – skewed hooks	1.26	0.057	<0.0001
		Stability tanks	1.09	0.027	<0.0001
		Primary production index, squared	0.99	0.001	<0.0001
		Survey index	1.10	0.004	<0.0001
		Catch mass of other species	0.96	0.007	<0.0001
Haddock	4314	Mean CPUE	0.025	0.002	<0.0001
		Swivel line – skewed hooks	1.51	0.065	<0.0001
		Stability tanks	1.34	0.035	<0.0001
		Primary production index, squared	0.99	0.001	<0.0001
		Survey index	1.08	0.004	<0.0001
		Catch mass of other species	0.92	0.007	<0.0001

Note: Estimates of month and area effects are not included in the table but are indexed relative to 2002 and illustrated in Fig. 7.

realized cod catches. However, annual increases were higher during both the earlier management regimes, averaging ~1.5% in the TAC period and ~1.6% in the TM period, and the general impression is a gradual increase across the entire examined period. So, although to some degree undermining the intentions of the TAE system, the annual increases in hooks fished per day of the period are probably only to a limited degree a result of introducing the system. The observed increases in hooks set per day are rather a result of a continuous technological development in longline fisheries

Fig. 7. Estimates of monthly CPUE effects for cod (triangles) and haddock (dots) with standard errors.



(Hilborn and Sibert 1988; Whitmarsh 1990; Ward and Hindmarsh 2007) and only to a lesser degree a result of the shifting Faroese management systems.

The two regulation shifts of the period were, however, clearly mirrored in the targeting behaviour of the longliners. A marked shift from secondary to primary (high value) target species accompanied the transition from output to input control in 1996, and the opposite shift (from primary to secondary target species) was observed following the shift from input to output control in 1994. The observed response towards high-value target species following the TAE introduction is also reported by Jákupsstovu et al. (2007), but it is contradictory to one of the main assumptions and rationales behind the concept of effort management, that fishermen automatically switch target species according to relative stock availability. In the Faroese case, it seems that most fishers opportunistically target the most valuable species almost irrespective of abundance, a mechanism that is presumably enhanced by increasing prices with decreasing landings, and that could potentially result in stocks being fished to extinction (Rossiter and Stead 2003).

Substantial CPUE increases brought about by technological development were also found to have taken place during the examined period. GLM modelling of haddock CPUE variations demonstrated a factor 1.51 increase following full introduction of the swivel line and skewed hooks and a factor 1.34 increase following introduction of stability tanks. Cod CPUE increased somewhat less following the two introductions (a factor 1.26 and a factor 1.09, respectively). Such estimates of CPUE increase seem drastic, and their reliability might be questioned. The questionnaire survey, however, inferred from skippers' opinions a 34% increase in catchability (particularly for haddock) from the swivel line and a non-species-specific catchability increase of 21% from the skewed hooks, thereby supporting the 51% (haddock) and 26% increases (cod) in catchability estimated from the catch and effort data. Other investigations have also reported substantial fishing power increases from developments in hook designs (e.g., Quinn et al. 1985; Skeide et al. 1986; Ward and Hindmarsh 2007) and swivel line (Bjordal 1989), and Sainsbury (1996) estimates a general 30%–40% catchability increase in longline fisheries from improvements in hook design and swivel line. The average of questionnaire estimates for catchability increases from stability tanks was 13%, suggesting that the GLM estimate of 34% for haddock is less reliable than the estimate of 9% for cod.

A species-specific effect from the swivel line and skewed hooks was anticipated, as skippers in both interview schemes had stated that haddock catchability was particularly affected, and this was confirmed by the modelling results with the somewhat lower estimates for cod. It has been shown that cod and haddock behave differently when taking bait. Haddock is characterized by biting repeatedly and carefully at parts of the bait, whereas cod completely ingests the bait, thereby increasing the probability of being hooked (Løkkeborg et al. 1989). It is therefore not unlikely that the skewness of the hooks in combination with the easy mobility of the swivel line substantially increased the hooking success for the carefully biting haddock and had somewhat less effect on the hooking success for the much more aggressively biting cod. It is less straightforward to explain the species-specific CPUE influence from the stability tanks (a factor 1.09 for cod and 1.34 for haddock). One of the gains from stability tanks is a better performance of crew and machinery when baiting, setting, and hauling the lines, and possibly the above described differences in the biting behaviour makes the haddock more susceptible to, for example, improved baiting of the hooks. It is, however, difficult to logically or empirically validate such a marked difference in CPUE effects from stability tanks, indicating that confounding of variables in the GLM cannot be completely ruled out.

The PP index had a significant effect on both haddock and cod CPUE, with a 1% decrease in CPUE with each increase in the squared index value of 1. For cod, this was expected, based on the results of Steingrund et al. (2009), and the result for haddock confirmed the assumption of approximately similar influences from higher food abundance on the catchability of the two species to longlines on the Faroese Plateau. CPUE of both species was also significantly influenced by the index values of stock abundance (a factor 1.10 for cod and a factor 1.08 for haddock with each increase in index value of 1), which was also expected.

Seasonal differences in cod and haddock CPUE values were found, indicating that the vessels might separately target either of the two species. It is possible, to some degree, by choice of bait to more specifically target either cod or haddock (Løkkeborg and Björdal 1992), and the estimation of a significant negative "other species" effect (4% for cod and 8% for haddock CPUE) also casts some doubt on the assumption that the Faroese longline vessels in general conduct a combined cod and haddock fishery and indicates that the two species to some degree are targeted separately.

Management implications

According to the results described, great care should be taken when defining the metric with which to manage fishing effort in an effort-regulated system. Across the entire examined period, we found a substantial discrepancy (1.3% annually) in the temporal development of the presently used Faroese effort descriptor, fishing days, and the number of hooks fished per day, which is more directly linked to the fishing mortality. This problem of poor linkage between nominal and effective effort (and ultimately fishing mortality) is of general nature in the management of commercial fish-

eries, and the identification of better effort descriptors for the various vessel and gear types of commercial fisheries is subject to investigation (e.g., Marchal et al. 2007; Rijnsdorp et al. 2006; ICES 2009). In the case of the Faroese longliners, the number of hooks fished per day without doubt has a closer linkage to fishing mortality than does the number of days fished, and this most likely goes for longline fishery worldwide. Consequently, the use of quotas of yearly hooks rather than fishing days would reduce the current mismatch between intended and realized fishing mortality of the Faroese effort regulation. The question is, however, whether this alternative measure of effort is sufficiently operational in terms of administration and control of effort quotas to make the transition worthwhile. Presumably it is more appropriate to integrate this discrepancy between nominal and effective longline effort development in a general provision for fishing power advancement as seen for other fisheries managed by effort days (Cadwallader et al. 2000). Such a provision is actually part of the Faroese effort regulation, but the implemented reduction of fishing days of approximately 1.5% annually (Jákupsstovu et al. 2007) has been too small to counterbalance the actual fishing power increases and an excess initial effort allocation as detailed below.

The demonstrated unregistered increases in effective effort presumably explain part of the mismatch between intended and realized fishing mortality of the Faroese effort regulation. A larger part of the explanations is, however, that the initial as well as the current number of yearly fishing days allocated to the different vessel groups, including the longliners, is too high to ensure a cod spawning stock biomass above safe biological limits (Jákupsstovu et al. 2007; Baudron et al. 2010). This flaw of the Faroese effort regulation scheme is easily apprehended from the observed doubling of total yearly hooks set by the five longliners in response to the shift from the TAC to the TAE system, and it underscores the importance of the initial design, when new management systems are introduced.

Technologically driven fishing power increases of the Faroese longliners is another mechanism that appears to have played a very important role in the decline of particularly the Faroese haddock stocks. Combining the demonstrated CPUE effects from skewed hooks and swivel line and stability tanks with the annual increase in number of hooks fished per day results in a total fishing power increase of the period of 154% for haddock and of 72% for cod, corresponding to yearly increases of 9.0% and 4.3%, respectively. At first impression, such figures appear very high, but Marchal et al. (2002) have demonstrated annual fishing power increases in the Norwegian longline fishery of 18% for cod and 15% for haddock across a 19-year period, and the magnitude of the individual estimates of CPUE increases are supported by the values inferred from the questionnaire survey.

Accepting that the overall estimations of technologically driven fishing power increases are in the right order of magnitude, these results question the ability of effort regulation as a means to control fishing mortality in commercial fisheries. The task of achieving agreement between intended and realized fishing mortality appears difficult in the light of the substantial and intermittent fishing power increases from technological developments demonstrated in this and other studies (e.g., Robins et al. 1998; Mahevas et al. 2004). The heterogeneity of the technological challenges is not only restricted to the development of different technologies in different fisheries or different use of the same technologies in different fisheries (e.g., the use of sonar technology for fish finding in pelagics and for small-scale navigation in wreck fishing with gill nets), but also to species-specific effects of the same technology development in the same fishery as demonstrated for the skewed hooks and swivel line. Bearing this complexity in mind, and adding to it the need to also identify and predict catchability changes from factors such as targeting behaviour, skipper skills, technical interactions, compliance, contraction of the stocks, and environmental conditions, the lessons learned from this study point at favouring output control — and coping somehow with discards and misreporting — rather than attempting to manage fishing mortality levels through expected matching levels of fishing effort. In this consideration, recent successful experiments with fully documented fisheries enabling the transition from landing quotas to actual catch quotas (Dalskov et al. 2009) have played a part.

Regardless of the pros and cons of input-oriented fisheries management, it was a little surprising that the technological driven mechanisms anticipated — and demonstrated — to have increased fishing power and undermined the intentions of the Faroese effort regulation were induced only to a limited degree by the introduction of the system. The mechanisms were already in progress, and at a higher speed, during previous management regimes. So, although compromising the intentions of the effort regulation, none of these mechanisms appear to be a direct reaction to the regime shift. More likely, the fishing power increases are results of a continuous process of technological development in commercial fisheries — a development that is presumably driven mainly by economic and technological processes and only to a lesser degree by whatever management system is in force.

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Environmental determinants of threespine stickleback species pair evolution and persistence

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Abstract: Threespine stickleback (*Gasterosteus aculeatus*) species pairs are found in four watersheds in southwestern British Columbia, Canada, and are listed as Endangered under the federal Species at Risk Act. Their origin is thought to be through a double-invasion process followed by character displacement; however, this hypothesis does not consider whether speciation is dependent on unique environmental factors necessary to support two species with separate habitat and resource requirements, which may be essential both for their evolution and persistence. To test whether species pair lakes have unique attributes, we compared abiotic and biotic factors of species pair lakes to lakes with only a single population of stickleback. There were no clear environmental differences between species pair and non-species pair lakes, but species pairs were only present in lakes with low fish species diversity, suggesting that evolution and persistence of species pairs requires a simplified fish community. Our study suggests that colonization history rather than unique lake attributes (related to either physical habitat or trophic resources) facilitated the evolution of stickleback species pairs and that the fish assemblage in lakes may affect resource availability and speciation potential as strongly as the limnological attributes of the lakes themselves.

Résumé : Les paires d'espèces d'épinoches à trois épines (*Gasterosteus aculeatus*) se retrouvent dans quatre bassins versants du sud-ouest de la Colombie-Britannique, Canada, et figurent sur la liste des espèces menacées d'après la loi fédérale sur les espèces en péril. On pense que leur origine s'explique par un processus de double invasion suivi par une divergence phénotypique; cependant, cette hypothèse ne considère pas si la spéciation dépend de caractéristiques uniques du milieu nécessaires pour le maintien de deux espèces avec des besoins différents d'habitat et de ressources, qui peuvent être essentielles tant pour leur évolution que pour leur persistance. Afin de tester si les lacs qui possèdent des paires d'espèces ont des caractéristiques uniques, nous avons comparé les facteurs abiotiques et biotiques des lacs à paire d'espèces avec ceux des lacs à une seule population d'épinoches. Il n'y a pas de différence environnementale claire entre les lacs avec ou sans paires d'espèces, mais les paires d'espèces ne sont présentes que dans les lacs à faible diversité de poissons, ce qui laisse croire que l'évolution et la persistance de paires d'espèces exigent une communauté simplifiée de poissons. Notre étude fait penser que c'est l'histoire de la colonisation plutôt que les caractéristiques uniques du lac (reliées à l'habitat physique ou aux ressources trophiques) qui a facilité l'évolution des paires d'espèces d'épinoches et que le peuplement des poissons dans les lacs peut affecter la disponibilité des ressources et le potentiel de spéciation aussi fortement que les caractéristiques limnologiques des lacs eux-mêmes.

[Traduit par la Rédaction]

Introduction

The evolution of closely related, but trophically differentiated, pairs of species of freshwater fishes is a repeated occurrence in north temperate lakes and is usually attributed to ecological radiation within depauperate fish faunas (Robinson and Wilson 1994; Taylor 1999). Recently evolved taxa are of great value to understanding the processes of evolution and are often endemics with restricted distributions and therefore are of particular conservation concern (Schluter 2000). Understanding the processes that lead to species differentiation is a central goal of evolutionary biology, but may also provide essential information for species management. From a conservation standpoint, the limnological conditions that are required for species differentiation may also be essential for continued persistence, and alteration of limnological attributes has been associated with the irreversible collapse of several recently evolved species into hybrid swarms (Todd and Stedman 1989; Seehausen et al. 1997; Taylor et al. 2006).

A possible precondition for the evolution of trophically differentiated lacustrine species is their occurrence in adaptive landscapes with unusual limnological attributes (e.g., an unusual type of habitat(s) with a unique range of water chemistry or trophic conditions, referred to here as the "environmental constraints hypothesis"). Alternatively, there may be nothing intrinsically unique about the characteristics of water bodies where species pairs evolved, and their presence in particular lakes may be due solely to random colonization events or landscape filters that limited colonization and fish faunal diversity (McPhail 1993; Poff 1997; Jackson et al. 2001: the "his-

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torical contingency hypothesis"). These contrasting scenarios have different conservation implications, with the first suggesting that maintaining unique limnological attributes (e.g., water quality, habitat, or specific trophic resources) is paramount for species persistence, whereas the second suggests that maintaining low fish faunal diversity and preventing the introduction of invasive taxa are higher priorities.

Several studies have demonstrated that the environment can facilitate differentiation of species. Well-known examples include the cichlids of East African lakes (Seehausen et al. 2008), Darwin's finches (Grant and Grant 2002), and *Anolis* lizards from the Caribbean (Losos et al. 1997). In the context of north temperate fish diversity, it was recently suggested that limnological attributes of lakes can influence the evolution of lake whitefish (*Coregonus clupeaformis*) species pairs, which show a higher degree of differentiation into limnetic and benthic ecotypes in lakes with reduced habitat and prey availability, presumably as a consequence of greater intraspecific competition for resources (Landry et al. 2007; Landry and Bernatchez 2010).

Sympatric limnetic and benthic threespine stickleback (*Gasterosteus aculeatus*) species pairs are of major conservation concern; they are listed as Endangered under Canada's federal Species at Risk Act and are an ideal system for exploring the conservation implications of speciation processes (McPhail 1994; Schluter 2000). Sympatric pairs of threespine stickleback at one time occurred in seven lakes in five watersheds in the Strait of Georgia, southwestern British Columbia (Schluter and McPhail 1992; Gow et al. 2008). Over the last 10 years, species pairs in two lakes have gone extinct probably owing to interactions with invasive species (Hatfield 2001; Taylor et al. 2006). In each of these lakes, stickleback occur (or once occurred) in benthic and limnetic forms and are adapted to different microhabitats (reviewed in McPhail 1994). Benthic sticklebacks have robust bodies with few, short gill rakers and feed on benthic macroinvertebrates in the littoral zone, while limnetic sticklebacks have a streamlined body form with many, long gill rakers and feed on zooplankton in the limnetic or pelagic zone (Larson 1976; Schluter and McPhail 1992).

The species pairs are thought to have originated through the double invasion of fresh water by a marine ancestor stickleback, with the first wave of invaders becoming the benthic form and the second wave of invaders the limnetic form (Schluter and McPhail 1992; McPhail 1993; Taylor and McPhail 2000). Although a double invasion scenario may have contributed to the evolution of sympatric species pairs, many other lakes in the region that were susceptible to more than one marine transgression contain only a single species of stickleback (non-species pair lakes). The fact that only seven lakes contained species pairs out of the multitude of lakes in the region suggests that these lakes are physically or biologically unique in some way that promoted the evolution of sympatric species. Given that benthic and limnetic sticklebacks show clear differences in habitat selection and resource use, any lake with species pairs will be required to meet the habitat needs of both species (i.e., contain both littoral and pelagic zones) as well as contain sufficient resources to support them (i.e., benthic macroinvertebrates and zooplankton). As such, under the environmental constraints hypothesis, there should be a clear difference in habitat types between species pair and non-species pair lakes, with large relative littoral and pelagic areas in species pair lakes, as well as high macrophyte abundance and a wider range of resource availability (i.e., higher plankton and benthic invertebrate biomass) than non-species pair lakes.

Alternatively, the existing biota of a habitat may play a more dominant role in subsequent evolution of communities (e.g., Shmida and Wilson 1985; Buckling and Rainey 2002; Johnson and Stinchcombe 2007). In fact, Vamosi (2003) compared six physical attributes of species pair and non-species pairs lakes, and while observing no significant difference in lake attributes, he found that they differed in their fish community. Stickleback species pair lakes contained only stickleback and coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), while most non-species pair lakes contained both of these fishes in addition to other fish species. Other fish species may act as predators and (or) competitors with stickleback, and resource depletion by other species could reduce the scope for specialization leading to speciation. Although Vamosi's (2003) study was important in highlighting the role of the fish community in stickleback species pair ecology, he characterized lakes largely in terms of physical habitat attributes, which represent only a subset of the axes that constitute the Grinellian niche of an organism (Guisan and Thuiller 2005; Soberón 2007). Species can also differentiate strongly along nutrient and resource gradients (Leibold 1989), and biological resources (e.g., abundance of benthic or planktonic invertebrates) represent an additional suite of adaptive axes (Svanback and Bolnick 2007; Landry and Bernatchez 2010) that were unexplored in earlier analyses. In particular, the chemical or nutrient characteristics of lakes, extent of aquatic macrophytes, and trophic resource availability are three key factors that have recently been implicated in evolution and persistence of species pairs in sticklebacks and other taxa (e.g., Landry et al. 2007; Landry and Bernatchez 2010). These additional lake attributes are critical to examine, as they are directly relevant to the biology of sticklebacks, their prey base, and the identification and delineation of habitat features potentially crucial to the persistence of species pairs.

In this study, we include all known species pair lakes, including an additional lake (Little Quarry Lake; Gow et al. 2008) discovered since Vamosi's (2003) study, and test the idea that limnetic and benthic stickleback species pairs have evolved because of unique abiotic and biotic factors that are shared amongst species pair lakes, but are absent in non-species pair lakes (i.e., the environmental constraints hypothesis). To test this hypothesis, physical, chemical, and biological variables were compared between lakes with and without species pairs. If species pair lakes group separately from all other lakes, this would support the hypothesis that a unique combination of environmental variables in these lakes were important determinants of stickleback species pair divergence. No difference in environmental attributes between species pair and non-species pair lakes would be consistent with the ideas that the evolution of species pairs was driven more by historical factors including, but not necessarily limited to, double invasion of a random subset of lakes, and (or) that differences in fish community structure are more important than biophysical–chemical properties of lakes in influencing the evolution of species pairs of threespine stickleback.

Table 1. Species pair and non-species pair lakes sampled.

Lake	Latitude (°N)	Longitude (°W)	Elevation (m)	Distance to sea (m)	Fish assemblage
Species pair lakes					
Balkwill	49.44	124.35	78	5000	S + C
Emily	49.44	124.32	31	3150	S + C
Paxton	49.42	124.31	97	3840	S + C
Priest	49.44	124.33	78	4720	S + C
Little Quarry	49.39	124.06	53	285	S
Enos	49.16	124.09	53	1500	S (hybrid swarm) + C
Hadley	49.29	124.17	61	1330	C + Bu (S extinct)
Solitary lakes					
Cranby	49.41	124.30	72	2530	S + C
Ambrose	49.43	124.01	59	940	S + C + Sc
Brown	49.44	123.54	49	496	S + C + Sc
North	49.44	123.58	45	945	S + C + Sc
Stowell	48.46	123.26	77	1400	S + C + R
Weston	48.47	123.25	69	1890	S + C + R
Chemainus	48.54	123.45	83	2650	S + C + R + B

Note: B, smallmouth bass (*Micropterus dolomieu*); Bu, brown bullhead (*Ameiurus nebulosus*); C, coastal cutthroat trout (*Oncorhynchus clarkii clarkii*); Sc, prickly sculpin (*Cottus asper*); S, threespine stickleback (*Gasterosteus aculeatus*); and R, rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Study lakes

Fourteen lakes in southwestern British Columbia containing threespine stickleback were sampled during this study (Table 1). Seven of the sampled lakes contain, or have historically contained, threespine stickleback species pairs (SP) and the other seven lakes contain a solitary population of threespine stickleback (non-species pair, NSP; Table 1) in addition to other fish species. All known SP lakes were sampled; NSP lakes sampled were between 45 and 100 m in elevation and ≤5000 m distant from the sea because this is the range of elevations and distances encompassed by SP lakes (and therefore should have the same historical probability of being colonized repeatedly by a marine ancestor as the SP lakes). Elevation and distance to sea were chosen following McPhail (1993) and Vamosi (2003), with an estimated maximum 10% error in measuring these parameters. It has been found that all SP lakes contain only one other species of fish, coastal cutthroat trout (with the exception of Little Quarry Lake where construction of an outlet dam may have blocked access to spawning habitat). Thus, it would have been ideal to sample NSP lakes that contain only stickleback and coastal cutthroat trout; however, this was not possible because of the limited number of lakes with this specific, simplified fish community. Therefore, the group of NSP lakes sampled contained the following fish assemblages: (*i*) Cranby Lake — stickleback + coastal cutthroat trout, (*ii*) Ambrose, Brown, and North lakes — stickleback + coastal cutthroat trout + prickly sculpin (*Cottus asper*), (*iii*) Stowell and Weston lakes — stickleback + coastal cutthroat trout + rainbow trout (*Oncorhynchus mykiss*), and (*iv*) Chemainus Lake — stickleback + coastal cutthroat + rainbow trout + (introduced, nonnative) smallmouth bass (*Micropterus dolomieu*) (Table 1).

All lakes were sampled for water chemistry, trophic resources, and macrophyte abundance once between 6 July and 28 August 2008 and again at Paxton and Cranby lakes between 29 November and 1 December 2008 and 20–21 April 2009. Bathymetric surveys were completed at each lake between 6 July and 28 August 2008. Logistic constraints prevented replicate sampling at the same lake, but seasonal sampling at one SP and NSP lake (Paxton and Cranby lakes) showed minimal changes in water chemistry and other attributes between spring, summer, and fall (Ormond 2010). Although the primary goal of our study was to compare attributes of SP and NSP lakes, an ancillary objective was to collect baseline habitat data for future environmental monitoring for these endangered species.

Water chemistry attributes

We sampled water chemistry parameters that were related to nutrient and trophic status, including pH, conductivity, turbidity, dissolved oxygen, temperature, water transparency, chlorophyll *a*, dissolved organic carbon, total organic carbon, coloured dissolved organic matter, dissolved inorganic carbon, total nitrogen, total phosphorous, alkalinity (total as $CaCO_3$), total suspended solids, and total dissolved solids.

Turbidity samples were taken at a depth of 0.5 m and measured using a Lamotte 2020e turbidity meter. Surface readings of pH and conductivity were measured using a YSI WTW340i meter. Dissolved oxygen and temperature were measured using a YSI model 58 oxygen meter every 0.5 m of depth to create dissolved oxygen and temperature profiles of each lake. Water transparency was measured using a LI-COR model LI-250 light meter at 0.12, 0.5, 1.5, 2.5, and 3.5 m, and light extinction coefficient (k) values were calculated for each lake. For all other variables, water samples were taken within 100 m of the geographic centre of each lake at a depth of 0.5 m and analysed by Maxxam Analytics (Burnaby, British Columbia).

Zooplankton and benthic invertebrate abundance

At each lake, three vertical zooplankton tows were collected at the deepest area of each lake using a 30 cm wide Wisconsin plankton net with an 80 μm mesh. Depth of the tows varied with each lake to ensure they included the thermocline so as

to maximize the collection of vertically migrating zooplankton (Wetzel 2001). Samples were filtered through a 250 μm mesh and preserved in 5% formalin. Volume (V) of water filtered for each tow was calculated using the formula

$$V = \pi r^2 h$$

where r is the radius of the plankton net mouth, and h is the depth of the vertical tow. Sampling methodology followed Tonolli (1971). We did not include a correction for net filtering efficiency, as this was consistent across lakes and should not alter relative plankton estimates.

Benthic invertebrates were collected from both sediment and rock substrates to determine relative differences in benthic invertebrate resource availability between lakes. Three sediment and five rock samples were collected at each lake; fewer sediment samples were collected because sediment substrate was more homogeneous. Substrate sampling stations were chosen systematically to include multiple areas of each lake; sampling stations were chosen first by locating the appropriate substrate zone within each lake and then randomly choosing the location of samples within each zone. Sediment samples ($n = 3$) were collected by carefully scooping sediment from a known area (0.0075 m^2) into a 250 μm mesh net attached to the end of a pole and held against the lake bottom, filtering the sediment through the 250 μm mesh, and preserving the sediment in 5% formalin. Littoral rocks ($n = 5$; after Rosenfeld and Hudson 1997) were collected by carefully placing them into a 250 μm mesh net held next to the substrate before lifting the net out of the water and scrubbing the rock with a brush to wash off all invertebrates. Invertebrates were rinsed from the rock and net and preserved in 5% formalin. To express invertebrate abundance as a density, the surface area (m^2) of each rock was calculated based on rock length and width.

In the lab, zooplankton samples were divided into quarters or eighths depending on zooplankton density using a splitter so that each counted sample had not more than 2000 individuals. Accuracy of the split samples was assessed by splitting a sample and counting each subsample. The lengths of 30 individuals of each taxon in the subsamples were measured using a digitizing system (10× magnification; Roff and Hopcroft 1986), and all individuals in subsamples were counted. Cladocerans were identified to family or genus; copepods were identified as order Calanoida, order Cyclopoida, or as copepod nauplii. *Chaoborus* spp. were identified to genus, and mites were identified as subclass Acarina. Biomass for zooplankton was determined using length–weight regressions

$$\text{weight} = a \times \text{length}^b$$

where a is the intercept and b is the slope (from Culver et al. 1985 and Yan and Mackie 1987).

Invertebrate samples from rocks were not large enough to justify subsampling, whereas larger invertebrate samples from sediment were divided using a splitter. Samples containing 250 mL of sediment were divided into quarters, and samples of 125 mL of sediment were divided in half. Accuracy of splitting was determined using the same procedure as for zooplankton. Benthic invertebrates were identified to subclass, order, family, or genus, and individual length was digitized as described above. Biomasses for benthic invertebrates were calculated using length–weight regressions as published by Smock (1980), Sample et al. (1993), and Benke et al. (1999).

Macrophytes

Emergent and submerged macrophyte coverage was surveyed systematically throughout each lake. The area of macrophyte beds were measured using a range finder while canoeing around the perimeter of each lake. The range finder was used to estimate the distance from several points along each macrophyte bed to the lake shore. These distances were used to calculate the area of emergent and submerged macrophytes, which was expressed as a percentage of total lake surface area.

Bathymetry

The littoral area is often defined as either the portion of a lake that is less than 6 m deep or the threshold depth where sufficient light for plant growth reaches the lake bottom (e.g., Wetzel 2001; British Columbia Ministry of Environment 2009). For this study, we defined the littoral area as the portion of the lake that is less than 3 m in depth, because the majority of macrophytes are within the 3 m depth contour, and stickleback (both benthic and limnetic) typically breed at depths of less than 2 m (McPhail 1994; Hatfield and Schluter 1996). Therefore, the area of depth less than 3 m will contain the habitat structure that is most important for benthic sticklebacks and for reproduction of both species.

To determine relative littoral area, bathymetric data were collected along multiple transects of each lake using a Lowrance LMS-525C DF GPS Fishfinder. Relative littoral area (depth < 3 m) was extracted from the bathymetric data by uploading the UTM coordinates and corresponding depths onto georeferenced maps in ArcGIS (ESRI 2009) and fitting depth contours with the 3D Analyst tool of ArcGIS. Maximum depth was extracted from bathymetric data, while lake perimeter and surface area were extracted from georeferenced maps in ArcGIS.

Fish community

The fish communities of each lake were determined using archived data from the BC FishWizard (http://www.fishwizard.com), an online database that contains information on fish distributions in British Columbia), and from Vamosi (2003). For analysis of the fish community, exotic fish species (e.g., smallmouth bass, present in one NSP lake; and brown bullhead, *Ameiurus nebulosus*, present in one extirpated SP lake) were excluded, and therefore only stickleback, coastal cutthroat trout, rainbow trout, and prickly sculpin were used, since the objective was to determine whether there was a distinct historical fish community associated with the evolution of species pairs. Rainbow trout have been stocked in both Weston and Chemainus lakes (NSP lakes; British Columbia Ministry of Environment 2010) and, therefore, may not be native to those lakes; however, the fish community previous to this stocking is unknown, and as rainbow trout are native to the region (Scott and Crossman 1973), it is possible that they are native to these lakes. As such, we considered rainbow trout to be native fish in this study.

Data analyses

Student's t tests were used on each limnological variable to test for statistical differences between the mean values for

Table 2. Physical and chemical environmental variables used in statistical analyses (PCA–DFA).

	Species pair lakes							Non-species pair lakes						
	Balkwill	Emily	Little Quarry	Paxton	Priest	Enos	Hadley	Ambrose	Brown	Chemainus	Cranby	North	Stowell	Weston
Physical														
Perimeter (m)	2268	1091	2600	2277	4400	1609	1130	3200	1727	800	3280	1737	983	2117
Relative littoral area (%)	27.8	18	2.2	58.9	41.6	47.8	51.5	9.2	1.3	18.3	63.1	7.1	99.2	19.6
Surface area (m^2)	10.4	7	22	17.2	44.4	17.2	6.7	28.9	16.5	5.1	43.1	36	4.6	18.1
Max. depth (m)	15.8	14.5	20.8	28.7	18	11.9	11.7	32.8	17.6	10	13.3	15.6	7.2	11.8
Chemical														
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	0.6	1.2	0.9	0.8	1.7	2.9	3.3	2.1	<0.5	4.5	3.7	0.9	1.6	1.5
Dissolved organic carbon ($mg \cdot L^{-1}$)	6.9	4.9	3.3	5.7	6	5.4	6.4	3.5	0.7	5.1	9.1	1.7	5.1	6.1
Alkalinity (total as $CaCO_3$; $mg \cdot L^{-1}$)	35	110	3.7	130	85	47	17	6.7	4.8	21	47	13	29	31
Total organic carbon ($mg \cdot L^{-1}$)	5.7	4.9	3.2	6.5	5.8	5.7	6.7	4.7	2.2	5.5	8.3	3.2	4.9	6.1
Coloured dissolved organic matter (colour unit)	10	5	<5	5	5	5	30	5	5	15	10	2.5	10	5
Dissolved inorganic carbon ($mg \cdot L^{-1}$)	8.3	26.3	<0.5	29.4	21.2	10.8	6.1	2.9	2.2	5.2	10.2	4.5	5.3	5.4
Total nitrogen ($mg \cdot L^{-1}$)	0.2	0.24	0.1	0.24	0.33	0.32	0.23	0.15	0.07	0.29	0.29	0.1	0.28	0.36
Total phosphorus ($mg \cdot L^{-1}$)	0.006	0.005	0.004	0.01	0.006	0.006	0.007	0.007	0.004	0.009	0.011	0.005	0.007	0.009
Total dissolved solids ($mg \cdot L^{-1}$)	44	160	24	150	150	82	60	24	20	36	74	28	66	60
pH	7.91	8.18	6.7	7.7	8.3	7.82	7.18	7.3	6.75	6.8	7.53	7.1	7.56	7.34
Conductivity ($\mu S \cdot cm^{-1}$)	83	296	25	273	259	148	99	27	21	58	117	43	110	110
Turbidity (NTU)	–0.02	–0.15	–0.29	0.16	0.18	0.27	0.33	0.32	–0.21	0.36	0.28	–0.7	–0.13	–0.23
Water transparency (*k* value)	–0.73	–0.4	–0.41	–0.46	–0.46	–0.48	–1.05	–0.65	–0.23	–0.8	–0.83	–0.37	–0.71	–0.55
Dissolved oxygen epilimnion (% sat.)	96.5	94.4	99.2	90	94.1	92.5	92.2	98.9	95.1	76.7	96.7	108.6	92	100.3
Dissolved oxygen hypolimnion (% sat.)	33.8	38.1	63.2	26.9	14	36.3	18.6	35.1	67.9	8.7	3.5	50.3	29.9	26.9
Dissolved oxygen mean (% sat.)	59.2	95.5	84.1	65.4	61.7	79.8	63.7	64.7	84.3	62.9	65.4	99.2	90.8	95.6
Temperature epilimnion (°C)	21.6	22.3	22	22.1	22.6	23	22.8	22.3	21.2	22.2	22.3	21.7	22.7	23.6
Temperature hypolimnion (°C)	6.3	8.5	5.3	7.1	8	8.1	5.1	4.9	7	7.9	8.7	6.2	8.3	7.1
Temperature mean (°C)	11.1	13.8	10.9	13.4	13.7	15	11.7	10.2	12.2	14.6	15.9	13.4	16.1	15.2

Table 3. Biological environmental variables used in statistical analyses (PCA–DFA).

	Species pair lakes					
	Balkwill	Emily	Little Quarry	Paxton	Priest	Enos
Zooplankton biomass ($mg \cdot m^{-3}$)	67.7 (17.1)	3168.7 (244.0)	1.0 (0.2)	2.5 (0.8)	175.9 (77.2)	8.2 (0.7)
Invertebrate biomass in sediment ($mg \cdot m^{-2}$)	3030.1 (757.7)	7204.0 (3099.2)	949.0 (122.1)	3650.7 (864.2)	8279.1 (2860.1)	1514.5 (393.3)
Invertebrate biomass on rock ($mg \cdot m^{-2}$)	739.3	1600.6 (429.6)	291.4 (104.5)	1385.5 (307.9)	2105.6 (959.4)	538.8 (98.2)
Emergent macrophyte coverage (% of lake surface area)	0.6	6.7	1.5	22.9	16.4	0.1
Submergent macrophyte coverage (% of lake surface area)	0.6	20.6	0.5	7.6	0.9	0.1
Total macrophyte coverage (% of lake surface area)	1.2	27.4	2	30.5	17.3	0.2

Note: Where applicable, standard error is given in parentheses.

each lake group (SP vs. NSP) using the spreadsheet-based general statistics program PAST (Hammer et al. 2001). When tests for homogeneity of variances failed, we employed the Welch's t test in R (R Development Core Team 2008).

Principal components analysis (PCA) was used to see if there were broad differences between SP and NSP lakes. PCA is a common multivariate method that takes multiple correlated variables and extracts uncorrelated combinations of these variables called principal components (PCs). The majority of variation in the data is described by the first few PCs, thereby reducing the number of variables needed to describe the data while retaining most information. For this analysis, the correlation matrix was used as the variables were measured on different scales (Manly 2005). The Jolliffe stopping rule was used to retain all eigenvalues (PC axes) greater than 0.70. Although the Kaiser–Guttman method, which retains eigenvalues > 1.0, is one of the most commonly used methods, we used the Jolliffe rule, as it retains more PCs and therefore more information about the objects (Jackson 1993). We conducted t tests of the retained PC scores from physical, chemical, biological, and combined PCAs between SP and NSP lakes.

Following PCA, discriminant function analysis (DFA) was used to differentiate between SP and NSP lakes if the Hotelling's multivariate T^2 test indicated a significant difference in PC scores existed between SP and NSP lakes (Manly 2005). The PCAs and DFAs both were performed on the retained PC scores (selected by the Jolliffe stopping rule) using PAST for all variables combined and on physical, chemical, and biological variables separately (Tables 2 and 3). The discriminant analyses employed the "leave-one-out" procedure (leaving out whole lakes) to determine the accuracy of correct allocations of lakes into groups by the analysis (Manly 2005). The physical PCA–DFA included four variables, chemical PCA–DFA included 19 variables, biological PCA–DFA included six variables, and the combined PCA–DFA included 29 variables (Tables 2 and 3).

Invertebrate biomass on rock and sediment could not be collected for several lakes because one or the other substrate was absent. To avoid a reduced data set in ordinations because of missing values, these missing observations (for rock, $n = 5$; for sediment, $n = 2$) were predicted using linear regression of total nitrogen on invertebrate biomass (for rock, $y = 4650x - 190$ ($r^2 = 0.33$, $p = 0.05$); for sediment, $y = 12\,100x + 680$ ($r^2 = 0.26$, $p = 0.045$)). We had the a priori expectation that invertebrate biomass would increase as total nitrogen increased because nitrogen contributes to primary productivity (Wetzel 2001), thereby increasing food resources for benthic invertebrates (e.g., Blumenshine et al. 1997). Consequently, we conducted a one-tailed test for significance of these relationships.

The statistical program MCLUST (Fraley and Raftery 2003) was used in R to determine the most likely number of groups formed by the lakes for the combined data set only. MCLUST is a model-based clustering analysis where there is no a priori designation of the number of groups. Several models, where each model consists of a different number of groups of lakes from 1 through to the number of individual lakes ($n = 14$), are compared at the same time by MCLUST, which then identifies the best model for the data based on the Bayesian information criterion (BIC). The best model has the highest BIC and an associated number of lake group as well as characteristic dispersion of lakes within groups. Following the hypothesis that SP and NSP lakes will differ in their suite of environmental parameters, we predicted that the best model selected by MCLUST would have two lake groups: SP and NSP lakes. Only PC axes retained after using the Jolliffe stopping rule were used in the MCLUST analysis (PC axes 1–8).

The distribution of fish species across SP and NSP lakes was analysed using a single linkage (nearest neighbour) hierarchic cluster analysis using Jaccard's similarity index, which is commonly used for presence–absence data (Jackson et al. 1989; Manly 2005). Jaccard's index does not include negative matches, and therefore if a species is mutually absent from several sites, this absence does not contribute to similarity between the sites (Cairns and Kaesler 1969). This index is appropriate for our study because some fish species only occur in a few of the study lakes (e.g., prickly sculpin occur in only three of 14 lakes), and by using Jaccard's index, lakes will cluster by positive matches alone. Jaccard's index ranges from zero (no similarity) to one (complete similarity).

Results

Physical comparisons among lakes

PC1 and PC2 accounted for 55.7% and 26.9%, respectively, of the total variance of physical variables. Perimeter (–0.62), surface area (–0.58), and maximum depth (–0.49) showed strong negative correlations with PC1,

Non-species pair lakes							
Hadley	Ambrose	Brown	Chemainus	Cranby	North	Stowell	Weston
38.4 (3.8)	2.9 (0.2)	15.7 (1.3)	21.2 (1.6)	22.8 (4.2)	15.4 (1.9)	100.6 (21.5)	346.3 (51.9)
3465.4	1193.8 (388.5)	2368.2 (427.9)	1929.3 (616.9)	4192	1955.5 (876.2)	4069.2 (1708.3)	4472.8 (888.1)
185.0 (29.3)	47.3 (15.5)	135.3	1157.4	871.0 (275.3)	557.7 (128.6)	1111	1482.7
3.1	0.003	0.8	68.8	17.2	2.2	6.2	3
7.4	0.01	0.2	9.1	1.1	0.9	1.9	3.5
10.5	0.02	0.9	77.9	18.3	3.1	8.1	6.5

which contrasted large, deep lakes with smaller, shallow ones. Relative littoral area (–0.83) had a strong negative correlation with PC2, which contrasted lakes with high versus low littoral area; lakes with intermediate PC2 scores had a good mix of both littoral and pelagic habitat.

Both SP and NSP lake values occurred across the range of lake sizes (PC1) and amount of relative littoral area (PC2) with no obvious differentiation between SP and NSP lakes (Fig. 1*a*). Using the Jolliffe stopping rule, the first two PCs were retained. There were no significant differences in mean scores for SP and NSP lakes along PC1 ($t_{[12]} = 0.072$, $p = 0.94$) or PC2 ($t_{[12]} = 0.0015$, $p = 0.99$).

The Hotelling's T^2 test for differences in group centroids on PC axes (PC1 and PC2) was not significant ($p = 0.67$ and $p = 0.99$, respectively), so no DFA was attempted.

Chemical comparisons among lakes

Species pair and NSP lakes showed near significant differences in conductivity ($t_{[12]} = 2.30$, $p = 0.052$; Fig. 2*a*), alkalinity ($t_{[12]} = 2.07$, $p = 0.077$; Fig. 2*b*), dissolved inorganic carbon ($t_{[12]} = 2.24$, $p = 0.062$; Fig. 2*c*), and total dissolved solids ($t_{[12]} = 2.25$, $p = 0.056$; Fig. 2*d*). While SP lakes as a group had higher mean values for these variables than NSP lakes, it was not a definitive factor for discriminating SP lakes because Little Quarry Lake (a species pair lake) had one of the **lowest** values for all four of these water chemistry variables (Fig. 2).

PC1 and PC2 accounted for 43.5% and 24.7%, respectively, of the total variance in the water chemistry data set. PC1 showed a negative correlation with dissolved oxygen in the hypolimnion (–0.32) and positive correlations with total organic carbon (0.32), total nitrogen (0.30), and dissolved organic carbon (0.30). PC2 had a negative correlation with coloured dissolved organic matter (–0.32) and positive correlations with water transparency (0.35), alkalinity (0.33), dissolved inorganic carbon (0.32), conductivity (0.32), and total dissolved solids (0.32). While SP lakes cover a larger spread on PC2, they are not obviously differentiated from NSP lakes or restricted to any part of the ordination (Fig. 1*b*). PC3 was most strongly correlated with mean temperature (0.51), mean dissolved oxygen (0.45), temperature in the epilimnion (0.33), and temperature in the hypolimnion (0.31).

The Jolliffe stopping rule resulted in the first five PCs being retained. Across all five PCs, there was a significant difference in the mean scores between SP and NSP lakes for only PC3 ($t_{[12]} = -2.52$, $p = 0.027$). The Hotelling's T^2 test using the PC axes (PC1 to PC5) was marginally significant ($p = 0.054$), and the subsequent DFA classified the lakes correctly as SP or NSP lakes with an accuracy of 85.7% after using the leave-one-out method.

Biological comparisons among lakes

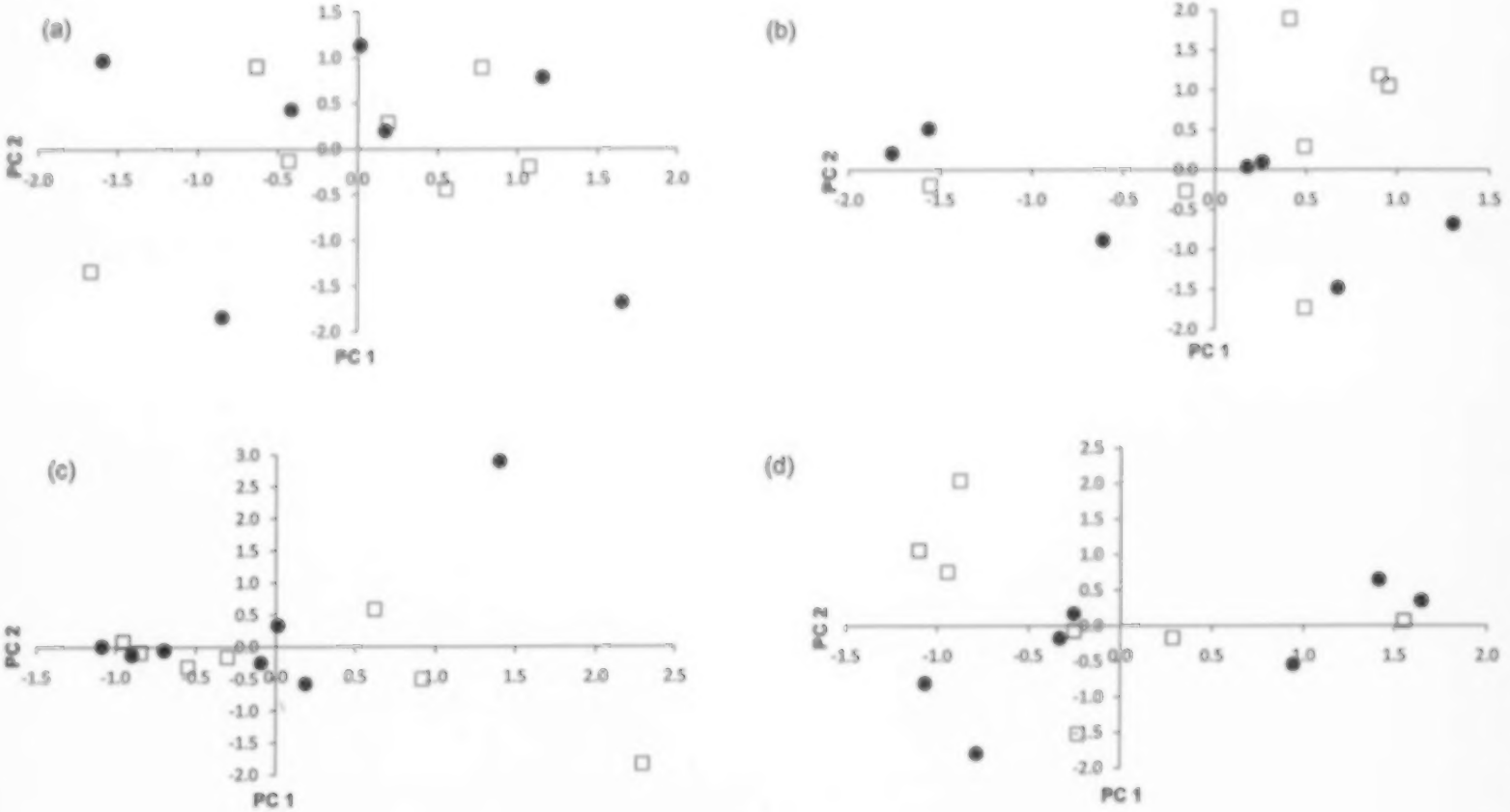
There was no significant difference in total macrophyte abundance between SP and NSP lakes ($t_{[12]} = -0.39$, $p = 0.70$). Macrophytes showed a range from 0.2% to 30.5% for SP lakes and 0.02% to 77.9% for NSP lakes (Table 3). SP and NSP lakes were similar in biomass (mg·m^{-2}) for both invertebrates in sediment ($t_{[12]} = 0.98$, $p = 0.35$) and invertebrates on rock ($t_{[12]} = 0.62$, $p = 0.55$).

For the PCA, PC1 and PC2 accounted for 52.9% and 28.6%, respectively, of the total variance. PC1 was correlated with submergent macrophytes (0.47), invertebrates on rock (0.45), total macrophytes (0.41), invertebrates in sediment (0.39), zooplankton (0.38), and emergent macrophytes (0.32). PC2 showed a strong positive correlation with emergent (0.62) and total macrophytes (0.51) and a negative correlation to zooplankton (–0.43) and invertebrates in sediment (–0.38). No obvious patterns were resolved between SP and NSP lakes (Fig. 1*c*). Following the Jolliffe stopping rule, *t* tests were performed on the first three PCs, and all were shown to be nonsignificant (all $p > 0.05$). Hotelling's T^2 test was not significant using the PC axes (PC1 to PC3) ($p = 0.66$), so no DFA was attempted.

Comparison among lakes with all variables combined

PC1 and PC2 accounted for 35.5% and 20.5%, respectively, of the total variance in the total data set of variables. PC1 showed a positive correlation with dissolved oxygen in the hypolimnion (0.27) and a negative correlation with total nitrogen (–0.27), total organic carbon (–0.26), and dissolved organic carbon (–0.25). PC2 was most strongly correlated with water transparency (0.32), chlorophyll *a* (–0.30), and coloured dissolved organic matter (–0.28). PC2 was also correlated with zooplankton (0.25), conductivity (0.24), dissolved inorganic carbon (0.25), alkalinity (0.24), and total dissolved solids (0.24). Again, no obvious patterns were apparent between SP and NSP lakes (Fig. 1*d*). Following the Jolliffe stopping rule, the first eight PCs were kept, but no significant differences between SP and NSP lakes were observed (all $p > 0.05$). Hotelling's T^2 test using PC axes

Fig. 1. Scatterplots of means scores along PC1 and PC2 for the principal components analysis (PCA) of physical (*a*), chemical (*b*), biological (*c*), and all variables combined (*d*). For the physical PCA (*a*), PC1 and PC2 account for 55.7% and 26.9% of the total variation, respectively. For the chemical PCA (*b*), PC1 and PC2 account for 43.5% and 24.7% of the total variation, respectively. For the biological PCA (*c*), PC1 and PC2 account for 52.9% and 28.6% of the total variation, respectively. For the PCA of all variables combined (*d*), PC1 and PC2 account for 35.5% and 20.5% of the total variation, respectively. Open squares denote species pair lakes, and filled circles denote non-species pair lakes.



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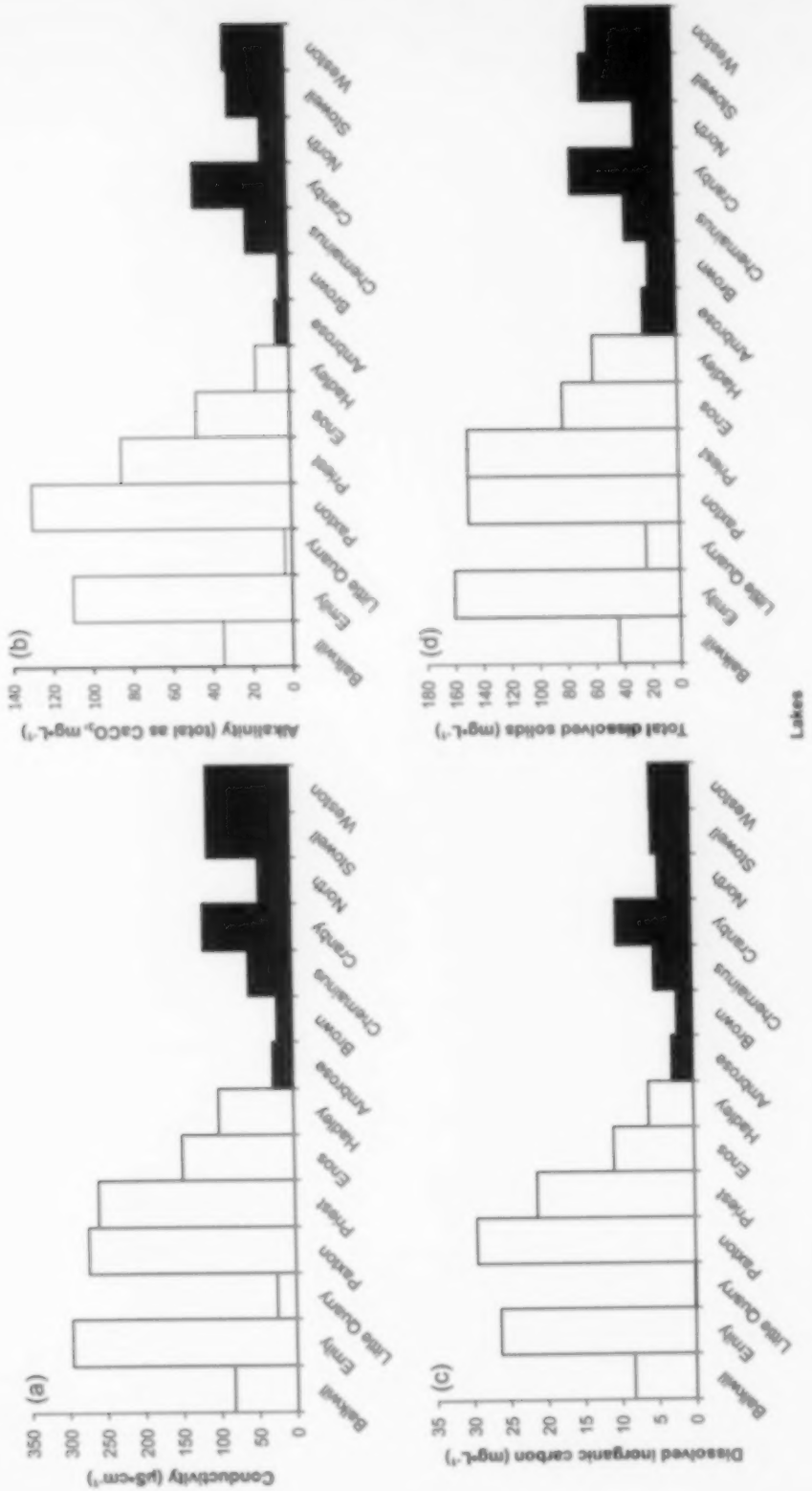
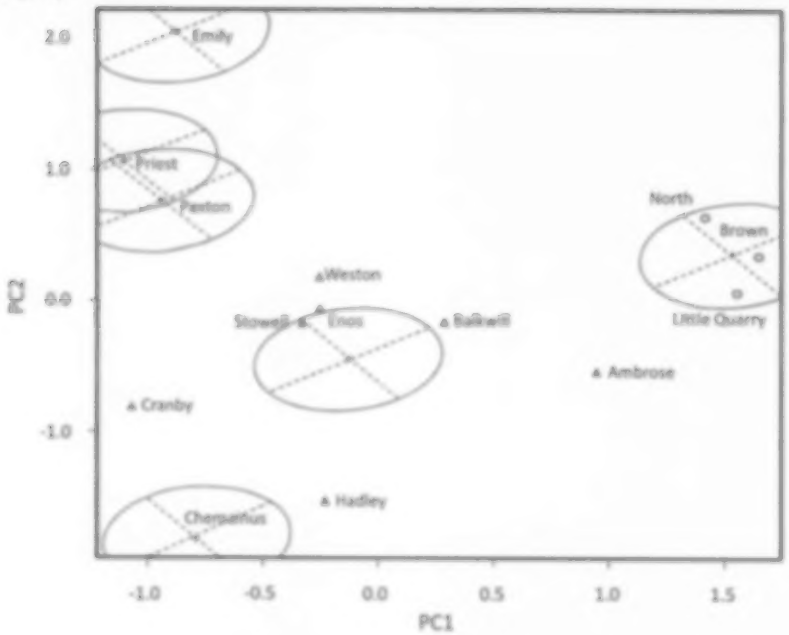


Fig. 2. Bar plots of conductivity (*a*), alkalinity (*b*), dissolved inorganic carbon (*c*), and total dissolved solids (*d*) for all species pair (open bars) and non-species pair lakes (filled bars).

Fig. 3. MCLUST analysis of species pair and non-species pair lakes along the principal component 1 (PC1) and 2 (PC2) axes. Each ellipse has equal variance. Weston, Stowell, Ambrose, Cranby, Enos, Hadley, and Balkwill lakes form one group; North, Brown, and Little Quarry lakes form a second group; Chemainus Lake forms a third group; Paxton Lake forms a fourth group; Priest Lake forms a fifth group; and Emily Lake forms a sixth group.



(PC1 to PC8) was also not significant ($p = 0.49$), and no DFA was attempted.

MCLUST analysis

The Jolliffe stopping rule was used to determine the number of PCs to retain ($n = 8$) for the combined data. A MCLUST analysis was performed on these first eight sets of PC scores from the combined data set of all physical, chemical, and biological variables (Fig. 1*d*). This analysis showed that the best model to explain the data included six components (i.e., groups of lakes) with equal ellipsoidal shapes and with equal variance within groups of lakes (i.e., lake groups are of equal volume, shape, and orientation). These six groups consisted of (1) Emily Lake; (2) Priest Lake; (3) Paxton Lake; (4) Chemainus Lake; (5) North, Brown, and Little Quarry lakes; and (6) Balkwill, Enos, Hadley, Cranby, Weston, Stowell, and Ambrose lakes (Fig. 3). Lakes did not appear to group according to SP vs. NSP lakes (Table 1), geography (Table 1), underlying geology (British Columbia Ministry of Energy Mines and Petroleum Resources 2004; Table 4), other measured limnological attributes (Tables 2 and 3), or fish community (Table 1, Fig. 4).

Fish community comparisons among lakes

The single linkage dendrogram formed four clusters of lakes according to fish species composition; cluster 1 includes the six SP lakes (Paxton, Emily, Balkwill, Priest, Enos, and Hadley lakes) and one NSP lake (Cranby Lake); cluster 2 includes the NSP lakes containing rainbow trout (Chemainus, Stowell, and Weston lakes), cluster 3 includes NSP lakes containing prickly sculpin (North, Brown, and Ambrose lakes), and cluster 4 includes the SP lake with no fish other than stickleback (Little Quarry Lake) (Fig. 4). Clusters 1, 2, and 3 were 66.7% similar to each other, while cluster 4 was 50% similar to all other clusters (Fig. 4).

Discussion

Differentiation between lake types

One of the key factors thought to be involved in rapid divergence of a lineage into different morphs is low competition from trophically similar species, which presumably allows a diverging population to expand into new and underutilized microhabitats (i.e., open niches; Schluter 2000). For example, Icelandic Arctic char (*Salvelinus alpinus*) can be found in lakes as either a single morph or with up to four different ecomorphs. The factors allowing–driving this divergence are thought to be a combination of resource heterogeneity and low species diversity and therefore low interspecific competition (Skúlason and Smith 1995; Knudsen et al. 2007). Indeed, the lake supporting four ecomorphs of Arctic char has a very heterogeneous environment and a depauperate fish community containing only Arctic char, threespine stickleback, and a small population of brown trout (*Salmo trutta*; Sandlund et al. 1987; Smith and Skúlason 1996). The importance of fish community diversity in the evolution of such species pairs can be seen in other fish taxa (Taylor 1999) as well as in other vertebrates such as lizards (Losos et al. 1997) and some birds (Grant and Grant 2002).

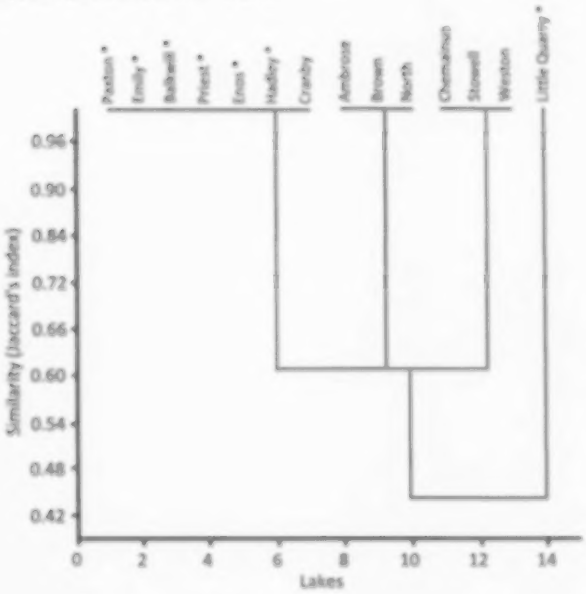
The repeated parallel evolution of benthic and limnetic pairs of stickleback species and their geographically clustered location suggests that there are ecological or historical factors

Table 4. Underlying rock types of all species pair and non-species pair lakes.

Underlying rock type					
Basaltic volcanic	Calcareous–alkaline volcanic	Granodioritic intrusive	Quartzdioritic intrusive	Undivided sedimentary	Limestone, marble, calcareous sedimentary
Balkwill (6)	Chemainus (4)	Ambrose (6)	Brown (5)	Enos (6)	Emily (1)
Cranby (6)		Hadley (6)	North (5)		
Paxton (3)		Little Quarry (5)			
Priest (2)		Stowell (6)			
		Weston (6)			

Note: Data indicates the lake name with MCLUST group number in parentheses. Rock type data were collected from British Columbia Ministry of Energy Mines and Petroleum Resources (2004).

Fig. 4. Cluster analysis (single linkage) on fish species composition in species pair and non-species pair lakes using Jaccard's similarity index for presence–absence data. Variables included were threespine stickleback, coastal cutthroat trout, prickly sculpin, and rainbow trout. Species pair lakes are denoted by an asterisk (*), while non-species pair lakes are unmarked.



specific to this geographic area that are necessary for stickleback trophic divergence (e.g., Schluter and McPhail 1992). As such, the goal of our study was to determine if species pair lakes are characterized by a specific suite of abiotic and (or) biotic lake attributes that are important determinants of divergence of the stickleback species pairs and, by inference, important for their persistence (the environmental constraints hypothesis). While specific historical events appear to be important in providing the opportunity for species pair formation (e.g., multiple invasions from the sea by marine stickleback, absence of barriers on inlet streams; Taylor and McPhail 2000), such historical factors (the historical contingency hypothesis) cannot be the only processes involved, as many more lakes were susceptible to multiple marine transgressions than those that support extant pairs of stickleback species.

Under the environmental constraints hypothesis, we expected that SP lakes would have a broader spectrum of prey resources than NSP lakes, thus providing more opportunity for sticklebacks to diverge into limnetic and benthic forms. Our results, however, showed a similar range of benthic and pelagic resources in SP and NSP lakes. The second expectation that the amount of littoral and pelagic habitat types would differ between SP and NSP lakes was also not supported, as both lake types had comparable amounts of littoral and pelagic habitat as measured by relative littoral area and macrophyte abundance. Our analysis, therefore, suggests that SP and NSP lakes have similar ranges in resource abundance, habitat structure, and other environmental attributes. By contrast, our data confirm Vamosi's (2003) observation that fish communities differed strongly between SP and NSP lakes; the former were found to contain stickleback only or a combination of stickleback and coastal cutthroat trout. Consequently, it appears that fish community is a major ecological determinant of whether species pairs are found in lakes in coastal British Columbia.

A greater abundance and diversity of fish species may increase both predation and competition, limiting the scope for stickleback diversification. While coastal cutthroat trout are both piscivorous and benthivorous as adults when they occur without other large predators, they can become more piscivorous when they coexist with rainbow trout (Nilsson and Northcote 1981). Consequently, in lakes where adult coastal cutthroat and rainbow trout are both present, stickleback may be preyed upon heavily in the pelagic zone by coastal cutthroat trout. To reduce the risk of predation it is common for small prey species, such as stickleback, to inhabit areas with more habitat structure, such as the littoral zone (e.g., Walters and Juanes 1993). This coupled with competition for zooplankton with juvenile coastal cutthroat trout and rainbow trout may have prevented stickleback from becoming fully pelagic in some lakes. In the littoral zone of NSP lakes, stickleback are susceptible to competition and predation from adult coastal cutthroat trout and rainbow trout, as well as adult prickly sculpin, which prey both on adult stickleback and their eggs (Pressley 1981). In addition, if competitors for either zooplankton (e.g., juvenile salmonids) or benthos (e.g., prickly sculpin) are more efficient at exploiting these resources, this would reduce resource abundance and decrease per capita resource availability to either benthic or limnetic stickleback, thereby lessening the intensity of any divergent selection. These factors may have prevented stickleback from becoming fully benthic and driven selection for a form that is intermediate in resource and habitat use in NSP lakes. As such, our results are in agreement with Vamosi (2003) and suggest that the relatively low levels to absence of interspe-

cific competition and predation were key factors that may have promoted diversification through character displacement by intraspecific resource competition. A similar case was presented by Landry et al. (2007), who compared environmental factors of highly differentiated lake whitefish species pair lakes with those of less differentiated species pair lakes as determined by differences in gill raker counts. Lake whitefish also differentiate as species pairs only in the absence of the lake cisco (*Coregonus artedi*, a direct pelagic competitor), while in our system stickleback species pairs coexist only with coastal cutthroat trout or when no other fish species are present. Thus, both in the lake whitefish and stickleback species pair systems, the fish community is depauperate and the lack of heterospecific competitors could promote expansion and persistence into new and open niches. Indeed, Lindsey (1981) originally suggested that the presence of sympatric benthic and limnetic forms of lake whitefish may be due to, and change in response to, the presence of other fish species such as ciscoes (whitefishes of the subgenus *Leucichthys*).

Landry et al. (2007) found that lakes with more highly differentiated lake whitefish species pairs had reduced habitat (lower hypolimnetic O_2) and zooplankton availability (lower density and length) and suggested that this would increase intraspecific competition for resources. Our study, however, found similar zooplankton availability (biomass) across SP and NSP lakes. It appears, therefore, that while differences in resource abundance may promote divergence in some lineages and areas, it does not appear to be a general factor determining where species pairs can occur, at least in stickleback. It is also possible that differences between lake groups seen by Landry et al. (2007) and Landry and Bernatchez (2010) could be a result of fish species altering attributes of the environment rather than the environment driving the divergences of fishes (e.g., Harmon et al. 2009; Bassar et al. 2010); that is, greater species differentiation could increase predation on zooplankton and benthos leading to increased production of phytoplankton and therefore increased transfer of detritus to benthic sediments, decreasing hypolimnetic oxygen.

Differences that were observed between attributes of stickleback SP and NSP lakes involved conductivity, total dissolved solids, dissolved inorganic carbon, and alkalinity, all of which were nearly significantly higher in SP lakes. Despite this apparent differentiation, both the highest and lowest, or next to lowest, values measured for each of these variables occurred in an SP lake (because of very low values in newly discovered Little Quarry Lake), thus making water chemistry unlikely to be the primary driver of species pair occurrence (i.e., while these data suggest that species pairs may be more likely to occur in more productive lakes based on water chemistry, it is not a necessary precondition for their evolution).

Our analysis assumes that the conditions necessary for species pair persistence (i.e. current conditions) are similar to those required for their initial speciation (i.e., historic conditions following deglaciation) and that present environmental conditions broadly reflect historic conditions. Although impossible to verify, this assumption seems reasonable; while it is likely that there has been some level of change over time (e.g., associated with terrestrial and aquatic vegetational succession with a watershed), limnological attributes like water chemistry are determined, in large part, by underlying bedrock. Recent increased levels of hybridization events within one species pair lake (Taylor et al. 2006) that has experienced environmental changes associated with invasive crayfish indicate that persistence of sympatric stickleback species is sensitive to environmental change. The continuing presence of species pairs in the other lakes, however, suggests that these lakes have not experienced rapid or severe change relative to historic conditions.

The observation of no detectable difference in planktonic or benthic invertebrate prey abundance between SP and NSP lakes also needs to be tempered by the limitations of our analysis. While we can be confident that there are no consistent major differences between lake types, it remains possible that subtle differences in invertebrate community structure may exist between SP pair and NSP lakes (e.g., Landry and Bernatchez 2010). Cause and effect, however, become increasingly confounded as effects become more subtle (i.e., small differences in invertebrate community structure could be generated by greater predation efficiency between ecotype assemblages in different lakes; e.g., Harmon et al. 2009), rather than differences in invertebrate communities reflecting divergent adaptive landscape that facilitated speciation.

Origin and persistence of species pairs

Our results suggest that although there are no obvious limnological features among those we measured that appear to be a precondition for species pair evolution, low species diversity of potential competitors and predators appears to be a key factor that can promote rapid population divergence (Schluter 2000). Again, however, not all lakes with low species diversity contain species pairs of stickleback. These results and observations imply that some aspect of the history of the lakes must be important to species pair formation. One such possibility is the notion that multiple invasions of the lakes (and how colonization is influenced by local geology, elevation, etc.) by different groups of sticklebacks has been an important historical factor influencing the distribution of species pairs (McPhail 1993; Taylor and McPhail 2000). In addition, the geographic history of the lakes is important when considering what other species have been able to access the lakes, which, as our results and those of Vamosi (2003) suggest, probably has been an important ecological factor in species pair formation.

Our results have important implications for the conservation of stickleback species pairs, all of which are listed as Endangered under Canada's Species at Risk Act. For instance, a critical factor in the persistence of species pairs would appear to be conserving the current species composition (i.e., keeping invasive species out and conserving coastal cutthroat trout), rather than forming specific abiotic environmental management criteria. The collapse of the Enos Lake species pair into a hybrid swarm following the appearance of the American signal crayfish, *Pacifastacus leniusculus* (Kraak et al. 2001; Taylor et al. 2006), and extinction of the Hadley Lake species pair following the introduction of the brown bullhead (Hatfield 2001) are examples of the devastating impact that invasive species can have on stickleback species pairs. That being said, habitat loss and degradation is the number one threat to freshwater fishes in Canada, and the effect of invasive species is often compounded by habitat

change (Dextrase and Mandrak 2006). In addition, in Enos Lake, the appearance of the American signal crayfish has been associated with the loss of aquatic macrophytes in the lake that provide important nesting habitat for benthic sticklebacks and whose loss is thought to be a major factor that drove the formation of the hybrid swarm (Taylor et al. 2006; Rosenfeld et al. 2008). Consequently, although we cannot point to one key habitat feature or combination of habitat features that sustain species pairs across all lakes, it is likely that major degradation or loss of habitat in existing SP lakes would compromise the persistence of benthic and limnetic sticklebacks. In addition, although SP lakes may not have unique limnological attributes relative to NSP lakes, water quality (e.g., dissolved oxygen, nutrients, turbidity) needs to be maintained within an appropriate range for the tolerance of the species (National Recovery Team for Stickleback Species Pairs 2007).

Key habitat with regard to stickleback species pairs includes not only the habitat needed to ensure survival of both benthic and limnetic species, but also the features of the environment that promote segregation in mating (Rosenfeld and Hatfield 2006; National Recovery Team for Stickleback Species Pairs 2007; Hatfield 2009). As SP lakes were not significantly different from NSP lakes with regard to their suite of environmental characteristics, the main management goal should be to maintain stickleback species pair lakes within their current values of all environmental parameters measured, particularly those that contribute to reproductive isolation (McPhail 1994; Gow et al. 2006), such as macrophyte abundance, which contributes to habitat heterogeneity and also affects nutrient load, water clarity, and availability of food resources. Substantial alterations to water quality or nutrients from their current levels could potentially be detrimental to the species pairs by changing the abundance and (or) type of food resources available or mate recognition cues (Boughman 2001), thereby increasing hybridization or altering selection pressures on limnetic and benthic species as well as their hybrids (Futuyma and Moreno 1988; Behm et al. 2010). Urbanization and human activities should also be regulated, as they can lead to sedimentation, eutrophication, and fluctuations of water levels, which alter the amounts of littoral and pelagic habitats available to stickleback and their resources. Finally, our study now provides baseline data for all known SP lakes to facilitate environmental monitoring of lake conditions and provides detailed environmental data for lakes that support healthy species pairs as well as for lakes that appear to favour an intermediate form (Enos Lake).

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Fishing activity of tuna purse seiners estimated from vessel monitoring system (VMS) data

Nicolas Bez, Emily Walker, Daniel Gaertner, Jacques Rivoirard, and Philippe Gaspar

Abstract: In the lack of fishery-independent information, catch per unit of effort (CPUE) is the conventional abundance index. In the case of the tropical tuna purse seine fisheries, a critical difficulty lies in the definition of an effective fishing effort, because fishermen use two different fishing modes (free swimming schools versus schools under fish aggregating devices) alternatively during the same trip. In this study, vessel monitoring system (VMS) data were used in an operational level to study and quantify the spatial dynamic of the tropical tuna purse seine fishing activity. A Bayesian state–space model allowed classifying VMS steps into three activities (fishing, tracking, and cruising), which were characterized by a small set of complementary spatial indicators. The dominant activity (49%) was clearly the tracking of tuna schools within areas of aggregations. A hierarchical spatial organization of the three fishing activities was also evidenced. Fishing strategies described by the triplets of proportions of time devoted to each activity and interpreted as compositional data were modelled by the sum of a vessel effect and a seasonal effect.

Résumé : En l'absence d'information indépendante des pêches commerciales, la capture par unité d'effort (CPUE) sert d'indice conventionnel d'abondance. Dans le cas des pêches tropicales de thons à la seine coulissante, une difficulté particulière est reliée à la définition d'un effort effectif de pêche, car les pêcheurs utilisent deux modes différents de pêche (sur des bancs à nage libre et sur des bancs associés à des dispositifs de concentration des poissons) en alternance durant la même sortie en mer. Dans notre étude, nous utilisons des données du système de surveillance des navires (VMS) à un niveau opérationnel pour examiner et mesurer la dynamique spatiale de l'activité de pêche tropicale aux thons à la seine coulissante. Un modèle état–espace bayésien a permis de classifier les étapes VMS en trois activités (pêche, poursuite et croisière) qui sont caractérisées par un petit ensemble d'indicateurs spatiaux complémentaires. L'activité dominante (49 %) est nettement la poursuite des bancs de thons au sein des zones de rassemblement. On a aussi pu dégager une organisation spatiale hiérarchique des trois activités de pêche. Les stratégies de pêche décrites par les triplets de proportions de temps consacrées à chaque activité et interprétées comme des données composées se modélisent par la somme d'un effet du navire et d'un effet de la saison.

[Traduit par la Rédaction]

Introduction

Traditional indirect and fisheries-dependent methods used to estimate stock sizes have either to be fed (surplus production methods) or tuned (cohort analyses) by an index of abundance. To be a reasonable surrogate for the unknown abundance, indices of abundance should behave over time like the true abundance. In the lack of fishery-independent information, such as scientific surveys, and assuming some strong working hypothesis (e.g., constant catchability), catch per unit of effort (CPUE) is the most used index of abundance in stock assessments. However, if this definition is simple in essence, its relevant evaluation may be unrealistic, as it needs the proper quantification of the amount of (effective) effort developed to catch fish. Nominal efforts are usually standardized to account for difference among vessels, areas, seasons, and years, but it was showed in many situations that final estimates remained close to nominal values (e.g., Soto et al. 2009).

For the case analyzed in the present paper, namely the French tropical tuna purse seiners operating in the Indian Ocean, the nominal fishing effort used to perform CPUEs is

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expressed as the time spent searching for tuna schools (i.e., the daylight time spent in the fishing grounds after removing the setting time and the inactive time: bad weather conditions, breakdown, etc.; Pianet 1998). Nominal fishing effort is then supposedly standardized accounting for different factors, such as the characteristics of the vessel, the season, and the fishing area (Pella and Psaropulos 1975; Laurec and Fonteneau 1979; Allen and Punsly 1984).

In the case of the tuna purse seine fishery, for which the detection of free-swimming schools depends upon visual clues (birds, radars, and sonars), the individual searching path of a vessel may be used to determine which proportions of the fishing day are effective in terms of fishing effort (Polacheck 1988). However, a developing fishing tactic used by tropical purse seiners in the world's oceans is to fish tuna schools associated with floating objects, which includes natural objects (e.g., logs, flotsams, etc.) or artificial man-made fish aggregating devices (FADs), usually bamboo rafts. Thousands of drifting FADs are regularly deployed in the Indian Ocean by the tuna purse seine fisheries (Moreno et al. 2007), and half of the world catch of tuna are captured around those FADs (Fonteneau et al. 1999). In the Indian Ocean, FAD fishing operations started in the late 1980s (Hallier and Parajua 1999) and developed since, but the definition of a proper fishing effort associated to the catch realized under FADs remains an open question. It must be stressed that fishing on free schools or on FADs are two different strategies characterized by different catches in terms of species composition and size categories. In this regard we assumed that the behaviour of the fishermen, and consequently the trajectories of the vessels, were strategy-dependent. The analysis of the individual vessel trajectories might then be helpful to detect what parts of the trips correspond to routes towards aggregative devices.

Because of a European regulation, European tuna purse seiners have been equipped since 2000 with vessel monitoring systems (VMSs). Based on previous studies performed in terrestrial behavioural ecology (Morales et al. 2004; Jonsen et al. 2005), we developed a method to infer the activity of purse seiners from their VMS trajectories (Walker and Bez 2010). The advantage of this later work was that the model predictions were validated by scientific observations made on board 10% of the fleet. These estimated fishing activities formed the basis of the present work and was summed up by a small set of complementary spatial indicators characterizing the spatio-temporal fishing activity.

Materials and methods

VMS and observers' data

Since 2000, the European Commission legislated that all European fishing vessels longer than 24 m should be equipped with a VMS. Using a global positioning system (GPS), positions of the purse seiners were registered every hour and transmitted to shore by satellite (Argos or Inmarsat). Being GPS positions, the data were accurate (error smaller than few tens of metres). Speeds (in knots; 1 kn = 1.853 km·h^{-1}) and turning angles (in radians, rad) between consecutive positions were readily calculated from VMS data. Data concerned only the western Indian Ocean French purse seiners exploiting yellowfin tuna (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*), and, even if this species is not targeted, bigeye tuna (*Thunnus obesus*). This fleet (between 14 vessels in 2006 and 17 vessels 2008) landed around 100 000 tonnes per year (Pianet et al. 2008). Given the schooling behaviour of tropical tunas and that fishers detect visually tuna schools at the surface of the sea, fishing activity occurs at day. Consequently only the daytime part of the individual trajectory was used (Fig. 1).

The VMS database used in this study included 141 489 GPS positions recorded daily from 1 January 2006 to 31 December 2008. However, data collected in January suffered a large level of misreporting (for uncontrolled reasons) and were thus excluded from the analyses. The full data set corresponded to 131 fishing trips performed by the 18 French purse seiners operating in the Indian Ocean. Hourly speeds depicted two clear modes corresponding, respectively, to stillness and full speed (around 12 kn; Fig. 2*a*). Meanwhile, turning angles showed a mode around 0 rad (straight line trajectories; Fig. 2*b*). These distributions were considered as a mixture of three distributions corresponding to different states (namely cruising, tracking, and fishing); each of them being characterized by specific speed and angle distributions. First, we expected a purse seiner to move quickly through abundance-poor areas and (or) towards fishing grounds (either indicated by the presence of other vessels or by beacon-instrumented FADs). These "cruising" phases were associated with high speeds and turning angles predominantly around 0 rad. It is worth mentioning that visual checking of tuna schools is constant on board fishing vessels during daylight, so that cruising phases have to be considered as effectively contributing to fishing effort (effective in terms of detection of new, rich patches). On the other hand, within areas where tuna schools are abundant (e.g., clusters or patches of tuna schools), skippers intensively try to track schools. In these "tracking" phases, the trajectory of the vessel is expected to be more sinuous, and consequently, apparent hourly speeds are expected to be smaller on average, even though the instantaneous speeds remain large, and turning angles should be widely distributed over the full 360° because of the numerous detections appearing in both sides of the vessel. Finally, purse seiners can remain still (i.e., immobile) for a while to set the net, to observe the behavior of school prior to setting, or to repair engine breakdown (this latter is considered to be seldom as skipper would tend as much as possible to postpone reparation until night). We thus considered that each of these three states contributed to the total amount of fishing effort with different but unknown respective weight.

An observers' database was used to complement the definition of the model structure used for estimating activities from VMS data, as well as to validate model predictions. The scientific observers' program conducted in the Indian Ocean is part of the European Data Collection Framework and targets 10% of the trips. Observers on board recorded the position of the vessel every hour or each time a change in speed or in turning angle (course) occurred. The beginning and the ending time of each fishing operation were also reported as well as the fishing mode (i.e., nonassociated school or free-swimming school and FAD school). Eleven trips (corresponding to 301 days at sea and 265 sets) were available for the analysis.

Fig. 1. Example of vessel monitoring system (VMS) trajectory (one vessel; August–October 2007). Continuous lines represent night data; circles represent daylight data. Strata 1 to 4 of the Indian Ocean Tuna Commission (IOTC) are represented. Inset shows location in eastern Africa.

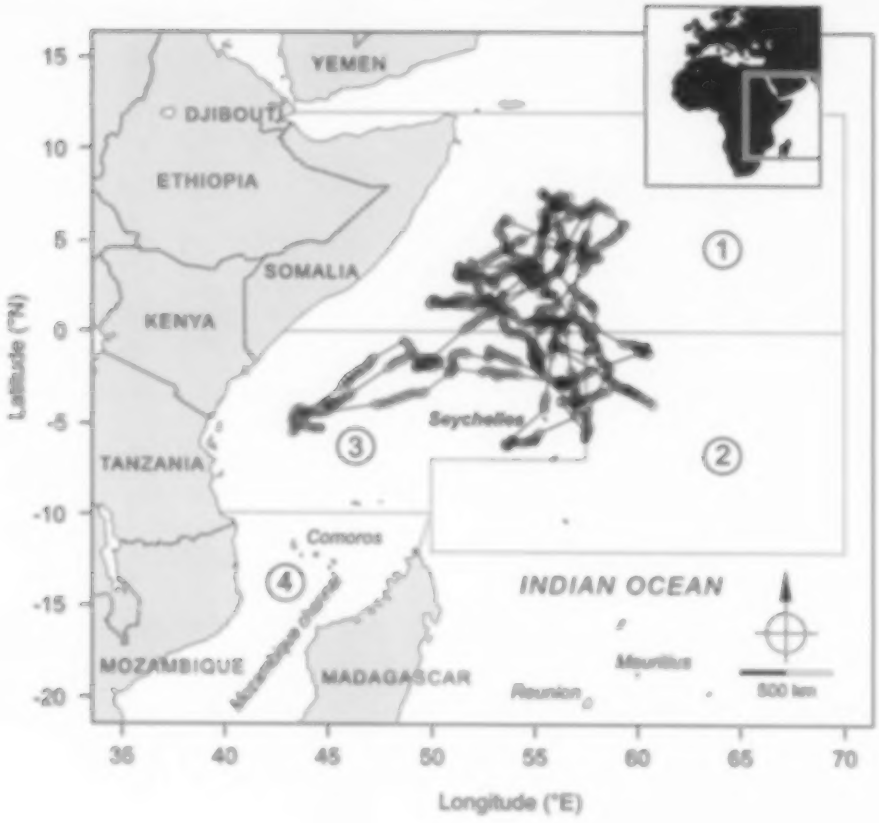
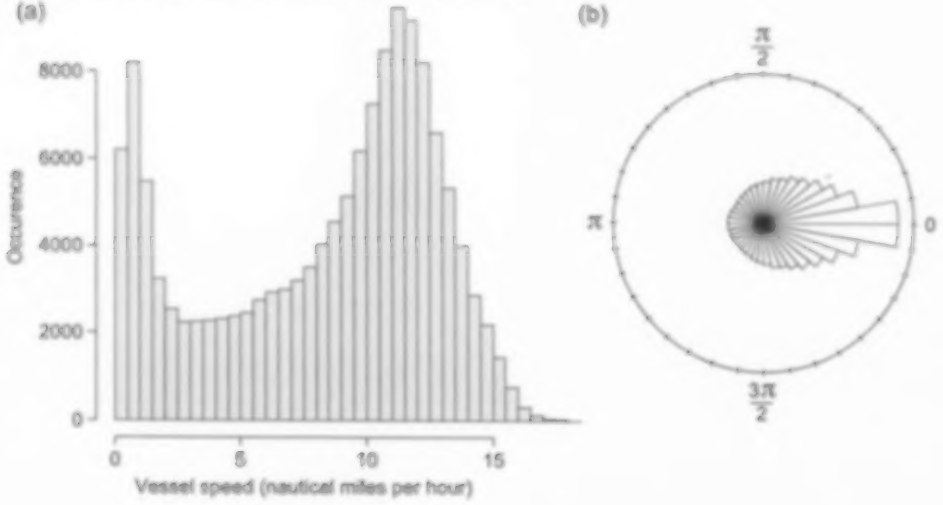


Fig. 2. 2006–2008 data of western Indian Ocean French purse seiners showing (*a*) histogram of hourly speeds (in knots; 1 kn = 1.853 km·h^{-1}) and (*b*) turning angles (in radians, rad) derived from VMS data (GPS positions).



Estimation of vessels' activities from the VMS trajectories

The model, for which a thorough description can be found in Walker and Bez (2010), consisted of a state–space model (Buckland et al. 2004; Royer et al. 2005; Patterson et al. 2008). Based on empirical evidence provided by observers' data, states were assumed to follow an order one Markovian process. States were inferred in a Bayesian framework (Gelman et al. 2004), knowing both vessel speeds and turning angles. Estimates corresponded to the state having the maximum a posteriori probability to occur.

The subset of VMS data corresponding to observers' data was extracted with the objective to tune some of the model parameters and, more importantly, to validate the model outputs before its application to the entire data set (97% of the fishing sets declared by observers were detected by the model; true-positive). Some sequences (e.g., three fishing sets of 2 h each only separated by a quarter of an hour) were viewed as one long sequence of six fishing steps.

The stops at sea (i.e., still phases that were estimated not to be fishing sets) represented 3% of the (daylight) time spent at sea by the studied fleet (Walker and Bez 2010). They were thus removed from the analysis.

Summary statistics of the components of the fishing effort at the fleet level

Model predictions were pooled at the fleet level by month and by geographical area (pixels of 0.2° × 0.2° or strata used by the Indian Ocean Tuna Commission (IOTC); see Fig. 1). Monthly time windows were chosen as a compromise to get enough data and to get relevant spatial coverages on the one hand and to have as much as possible small time windows to seek as much as possible synoptic views of the fishing effort spatial distributions on the other hand. The size of the pixels was chosen in reference to the lateral detection (~15 n.mi; 1 n.mi = 1.852 km) of tuna schools by visual inspection (binocular). Given this value, a 0.2° × 0.2° pixel was considered as exhaustively sampled as far as a vessel visited it. All results are given in hours spent in the states contributing to the total fishing effort.

Time series of the time spent by the French fleet in each type of fishing activities (fishing, tracking, and cruising) by month were produced either for the entire fishing grounds or by IOTC strata. Only the information coming from vessels operating at least 10% of the spatio-temporal window were considered.

As far as spatial characteristics are concerned, we used monthly maps of the model outputs aggregated (summed) at the fleet level and over 0.2° × 0.2° pixels for each activity (fishing, tracking, and cruising). Since it was not easy to compare simultaneously the 108 (36 × 3 = 108) raw maps, the main features of each map were summarized by using few dedicated statistics. First, the spatial extension of each activity was expressed in number of positive pixels. The homogeneity of the statistical distributions of the time spent by activity and by pixel was quantified by the Gini's index (1921) of the positive values; its variability over time was measured by the coefficient of variation (CV) of the 36 available Gini's indices. Gini's index equals 0 when all positively valued pixels have the same value, increases when there are a few patches of large values, and tends to 1 when one pixel value is much higher than all others. The analysis of each spatial distribution was complemented by the computation of an omnidirectional variogram (Rivoirard et al. 2000). The sets of the 36 standardized variograms by activity (i.e., empirical variogram divided by the sample variance) were represented by boxplots, allowing highlighting of dominant and temporally stable spatial features when they exist.

The two by two relationships between activities were summarized by Spearman's coefficients of correlation. This was complemented by the local index of collocation (Bez and Rivoirard 2000). This index, computed as a noncentered coefficient of correlation (mean product of noncentered variables divided by the root mean squares of each variable), is not impacted by zeroes. It is sensitive to the fact that large (versus low) values of each component occur in the same pixel. The local index of collocation equals 0 when one variable is null and the other is positive. It equals 1 when the two variables are identical or proportional. In addition to the correlation analysis, all omnidirectional cross-variograms were performed. They were standardized by the product of the standard deviations and represented by boxplots.

It is important to note that all the spatial statistics are dependent on the pixel size chosen for the analysis (support effect; Rivoirard et al. 2000).

Data analyses were performed with R and the package RGeoS (http://cg.ensmp.fr/rgeos).

Compositional data and fishing strategies at the vessel level

To account for temporal changes in the total time spent at sea, the time spent by each vessel and by month in each of the three activities was expressed as a percentage. Each triplet of proportions was interpreted as a fishing strategy. Mathematically speaking, each triplet is a composition (Aitchison 1982), that is, a set of values constrained by a closure relationship (the closure here was nothing but the fact that the proportions sum to one). The closure induces negative dependencies among the data that must be taken into account in the interpretation of graphical representations and analyses. Compositions are properly represented in simplex, here a triangle that is a trigone (three components) in a two-dimensional space (one degree of freedom missing because of the closure).

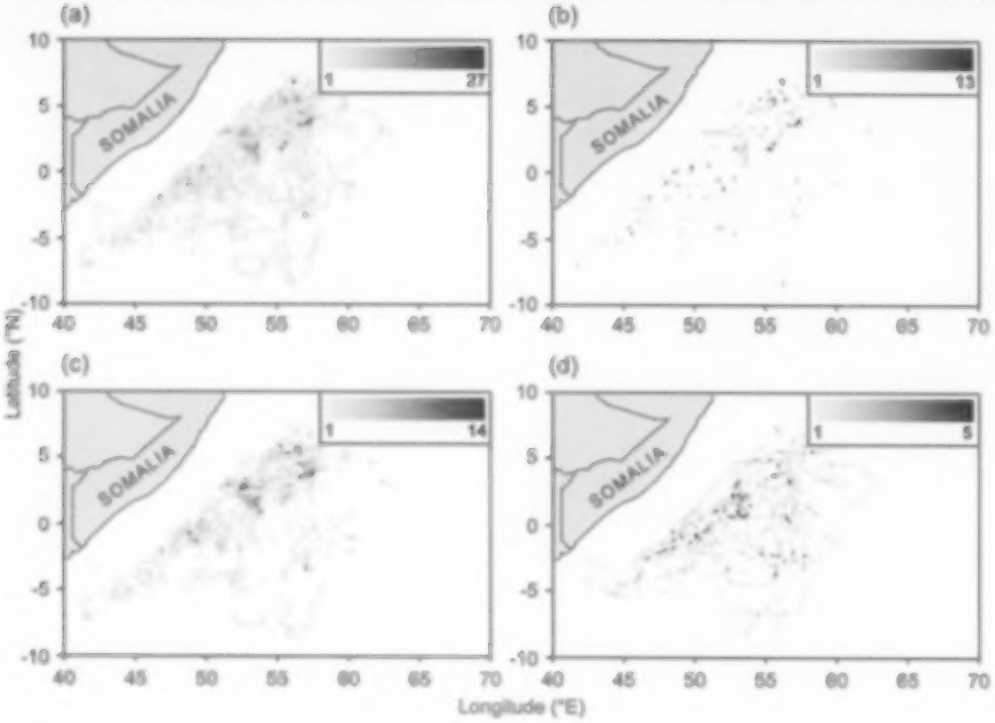
A log-ratio transformation allows projecting the data in a Euclidean space where all the usual statistical tools apply (Aitchison 1982). This transformation was used to explain the fishing strategies (i.e., the triplets of proportions) by the explanatory variables available in this analysis: month, vessel number, and strata. A linear model with all the simple effects and all the cross effects was applied. After selecting the statistically significant effects, these were back-transformed for a proper representation in the simplex. Analyses of the compositions were performed with the R package "compositions" (http://cran.r-project.org/).

Results

Fishing effort deployed by the fleet

Monthly distribution maps allowed a fine-scale analysis of the fishing effort (see Fig. 3 as an example). Over the whole period analyzed (2006–2008), the longest times ever spent by the French fleet by 0.2° × 0.2° pixel and by month were 90 h

Fig. 3. Spatial repartition of the fishing effort (hours) at the scale of 0.2° × 0.2° pixels. August 2007: (*a*) the time spent by the fleet irrespective of its activity representing the sampling effort distribution; (*b*) fishing operations (sets); (*c*) tracking phases; and (*d*) cruising phases.



(fishing), 71 h (tracking), and 10 h (cruising). The corresponding coefficients of variation were 4.2, 1.7, and 1.1, respectively.

The time spent in each type of fishing activity fluctuated around 1500 h per month for the fleet, with strong variations upon season and strata (Fig. 4). Overall, the percentage of this time spent at sea devoted to fishing, tracking, or cruising were 24%, 49%, and 27%, respectively (Fig. 4*a*). A clear spatio-temporal pattern emerged for each component of the fishing effort associated to the already known (Fonteneau 2010) seasonal displacement of the fleet connected to the tuna migrations (Figs. 4*b*–4*e*). As a matter of fact, the fleet, and thus the catch, follows tuna migrations over the western part of the Indian Ocean, shifting from strata 4, 2, 1, and 3 as time progresses over the year (Fonteneau 2010) and peaking in each of these areas in May, July, October, and December, respectively, with, however, fluctuations of dominant activities during these peaks.

The area where fishing took place was stable over time (CV = 0.23) and small (110 000 km^2 on average), while it was more variable (CVs = 0.32 for both) and large (426 000 and 405 000 km^2, respectively) for tracking and cruising (Fig. 5*a*). While the surface occupied was the same from month to month, the times spent inside these areas were not distributed similarly. Gini's index (Fig. 5*b*) showed similar levels for fishing and tracking activities with, however, a significantly smaller average value for cruising (Wilcoxon test; p value ≈ 2.10^{-5}); no clear temporal pattern emerged. Cruising activity depicted systematically smaller Gini's indices than fishing and tracking activities. A significant slight increase over time appeared, indicating that the cruising activity tended to be slightly patchier at the end than at the beginning of the studied period (2006–2008).

Monthly spatial structures of each activity (Fig. 6*a*) were strongly consistent over time (small expansion of the boxplots). This stability of the spatial structures of the vessels activities was observed despite large month-to-month geographical shifts of the fleet in the Indian Ocean (Figs. 4*b*–4*e*). The same spatial features were thus repeatedly observed for each of the three fishing activities considered whatever the location of the fleet in the western Indian Ocean. The spatial ranges happened to be about 0.6° (36 n.mi.) for the fishing activity and 1° (60 n.mi.) for the other two. The local heterogeneity of the spatial distributions was quantified by the (proportion) nugget effect of the empirical variogram. It was small for tracking (smooth maps on average) and large for fishing and cruising (rough maps on average).

At the resolution chosen for this study (i.e., monthly pixels of 0.2° × 0.2°), the coefficients of correlation were generally negative and small between cruising and fishing or tracking (Fig. 7*a*). It was significantly smaller between cruising and tracking than between cruising and fishing ($\overline{\rho}_{C,T}$ = –0.13; $\overline{\rho}_{C,F}$ = –0.04; Wilcoxon test; p value = 0.0052). Meanwhile it was positive and quite large for tracking and fishing ($\overline{\rho}_{T,F}$ = 0.55, CV = 0.23). The hierarchy between pairs was different when looking at the local co-occurrences (Fig. 7*b*). Fishing and tracking remained the most co-occurrent vessels' activities, but fishing and cruising were in strong opposition

Fig. 4. Monthly time series of the time spent by the French fleet in each type of fishing activity (fishing — open; tracking — grey; cruising — solid): (*a*) total area, (*b*) strata 4, (*c*) strata 2, (*d*) strata 1, and (*e*) strata 3. Boxplots of each set of values are represented on the right end side of each graph. The *x* axis represents months for the period 2006–2008.

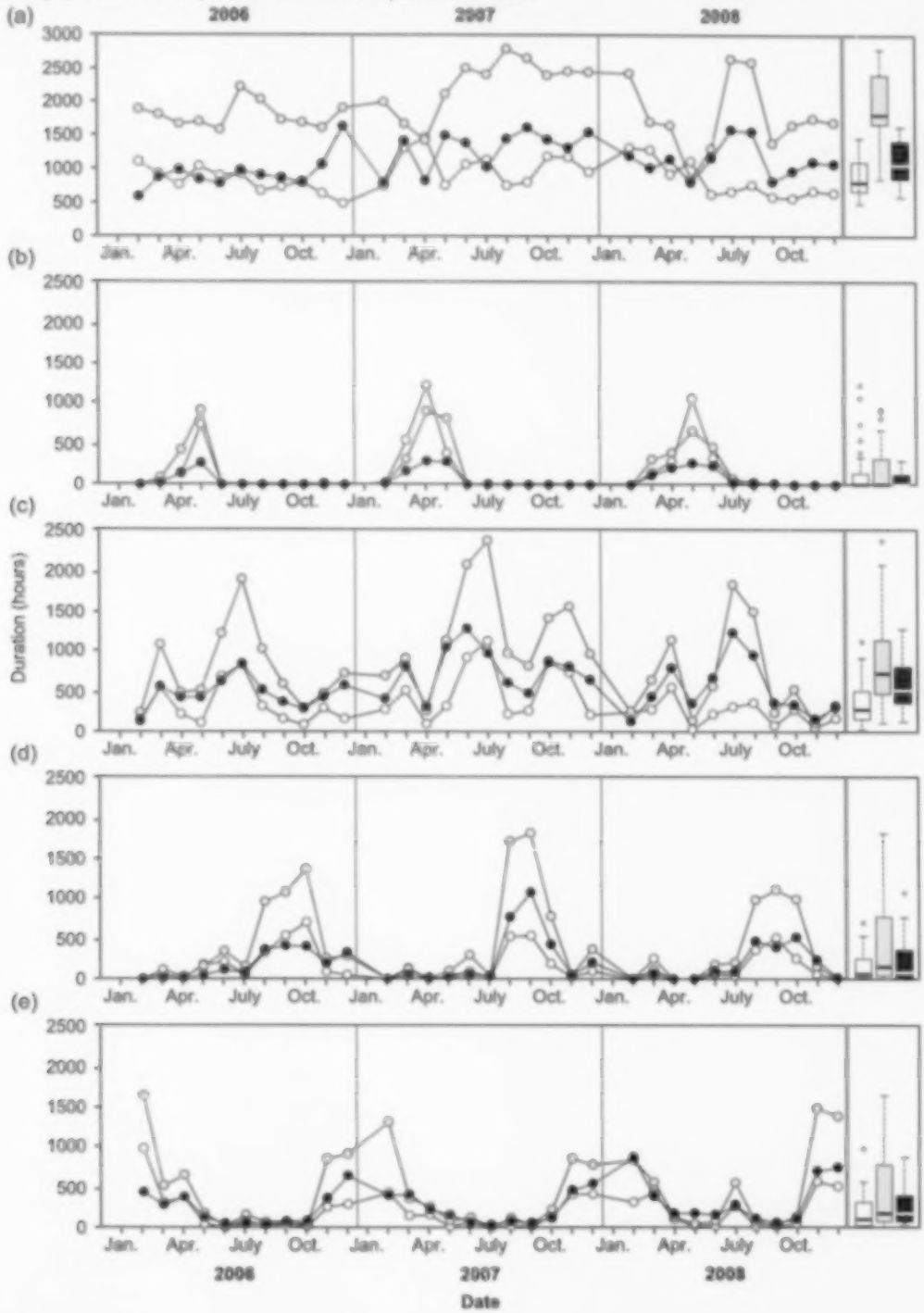
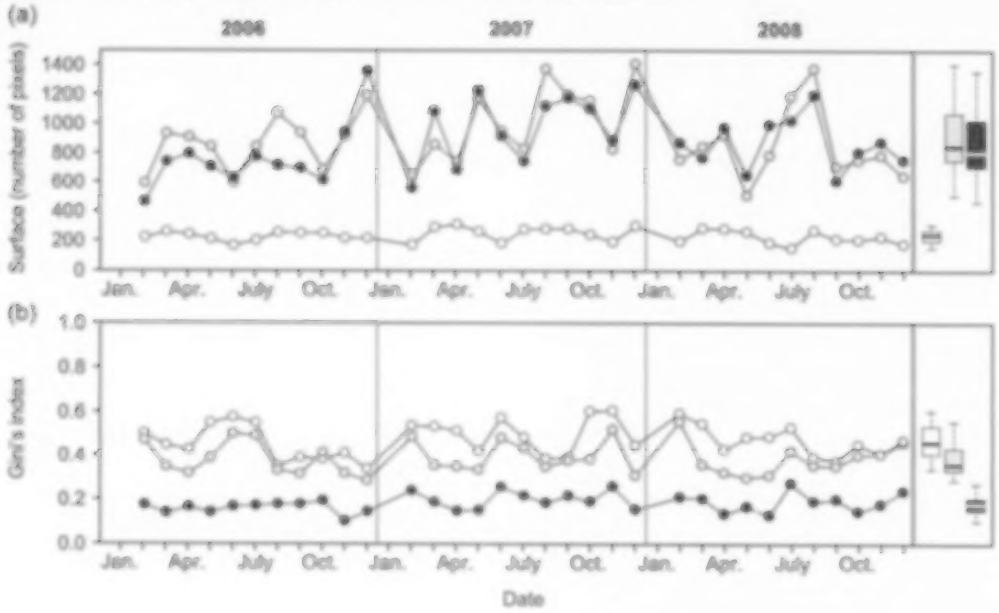


Fig. 5. Monthly monovariate summary statistics of the various fishing activities (fishing — open; tracking — grey; cruising — solid) aggregated at the fleet level: (*a*) area of presence (in number of $0.2° \times 0.2°$ pixels); (*b*) Gini's index. Boxplots of each set of values are represented on the right end side of each graph. The *x* axis represents months for the period 2006–2008.



with small local indices of collocation (LIC); the times spent fishing and cruising were not large or small, respectively, in the same time (i.e., in the same pixel).

The (standardized) cross-variograms between activities depicted spatial structures strongly consistent over time (Fig. 6*b*). Fishing and tracking covaried positively in space, that is, a positive increase of one variable being associated, on average, to a positive increase of the other, up to 0.6°–0.8° distance (~40 n.mi.). Fishing and cruising spatial patterns were very weakly correlated. Finally, tracking and cruising cross-variograms were, on average, negative, indicating opposition in their spatial covariation (one increasing when the other decreases) with no clear spatial range. Notice that contrary to the simple variograms, none of the three average cross-variograms got nugget effect. (The average values of the various summary statistics are recapped in Table 1.)

Fishing strategies

The proportions of time spent by activity, month, pixel, and vessel were not uniformly distributed over the entire triangle but rather concentrated around the centre of gravity (24% fishing, 49% tracking, 27% cruising). Tracking represented systematically more than 20% of the time spent at sea (Fig. 8).

After selecting out the strata and the cross effects that were not significant, the best linear model explaining the compositions of fishing activities was a sum of quarter effect and a vessel ID effect without interaction. The *p* values were 1.7×10^{-9} and less than 2.2×10^{-16}, respectively.

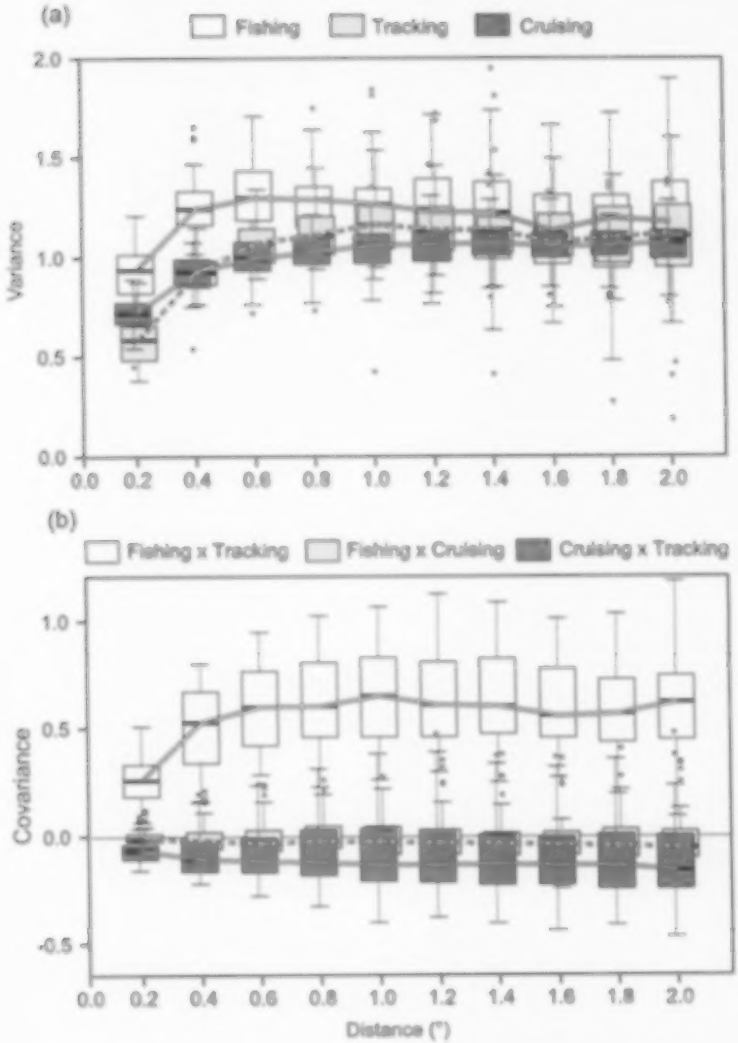
Once represented in the simplex, the quarter effect was parallel to the basis of the triangle associated with cruising. Fishing strategies changed significantly with quarter, and this change concerned essentially the allocation of time spent on fishing and tracking, the proportion of cruising activity being, on average, stable over time. Vessel ID also explained changes in fishing strategy, but orthogonally to trimester. Vessels realized, on average, the same proportion of fishing but differed significantly from the proportion of time they allocated to tracking and cruising. The two extremes were vessels whose strategies happened to allocate 40% and 65% of their time to the tracking of schools.

Discussion

VMS data for fisheries management

Defining a relevant fishing effort is a dilemma in the general case. CPUEs ought to be indices of abundance, but they are not until fishing effort measures properly the effective effort spent at sea to generate the catch. In the case of the purse seine tuna fisheries, in addition to the usual weaknesses associated to the definition of an effective fishing effort, the problem is particularly salient because purse seiners target different components of the tuna resource by using two different fishing modes (free-swimming schools versus logged schools under FADs) to which two different effective fishing efforts should be associated. Purse seiners are indeed practicing two different métiers simultaneously. In the absence of knowledge on fleet fishing strategies, crude fishing effort definitions, such as number of daylight hour minus the time spent effectively fishing, are traditionally used by the relevant tuna regional fisheries management organizations for stock assessments. The fact that this fleet is practicing two different métiers is indeed not properly addressed. One single and common definition of fishing effort is applied to all catches. In some cases, only free-school CPUEs of large yellowfins

Fig. 6. Monthly simple (*a*) and cross- (*b*) variograms of the various components of the fishing effort (fishing, tracking, cruising). Variograms were standardized by the experimental variances. Cross-variograms were standardized by the product of the standard deviations. Boxplots are based on monthly omnidirectional computations. They are slightly shifted horizontally to make them visible. The *x* axis represents the distance in degrees.



are considered relevant to follow the response of the stocks to the exploitation.

One explanation for this is that the spatio-temporal resolution of the information available in the logbook does not provide sufficient information on the vessel behavior to build any finer fishing effort. Observers' data do, at least in the context of the European Data Collection Framework, where one record must be done each hour at minimum and at any change of activity of the vessel. However, the observer program available in this fishery has a limited coverage (only 10% of the fleet), which precludes the use of these data for computing any relevant effort for the entire fleet. The present work demonstrated the interest in using VMS data at an operational level with the objective to quantify and localize three major components of the fishing effort. Fishing behaviors of tuna purse seiners and time spent in different fishing activities have already been studied from observers on board (Gaertner et al. 1999). This latter work studied in detail the chasing behavior of fishermen in the vicinity of tuna schools and in particular the phases when vessels decide to pursue schools or to set the seine. The authors also documented factors leading fishermen to change fishing zone, but this did not provide the means, at the fleet level, to move towards the definition of an effective fishing effort. The VMS data and the methodological framework developed to analyze them in a finalized manner (including combined with observers' on board data) provide, in our opinion, a unique opportunity to tackle the question of fishing effort.

Most of the VMS studies that are found in the literature have dealt with trawl fisheries (Lee et al. 2010; Rijnsdorp et

Fig. 7. Monthly bivariate summary statistics of the various fishing activities (fishing, tracking, and cruising: fishing × tracking — open; fishing × cruising — gray; cruising × tracking — solid): (*a*) coefficients of correlation; (*b*) local index of collocation (LIC); it equals 1 when large values occur in the same pixel. Boxplots of each set of values are represented on the right side of each graph. The *x* axis represents months for the period 2006–2008.

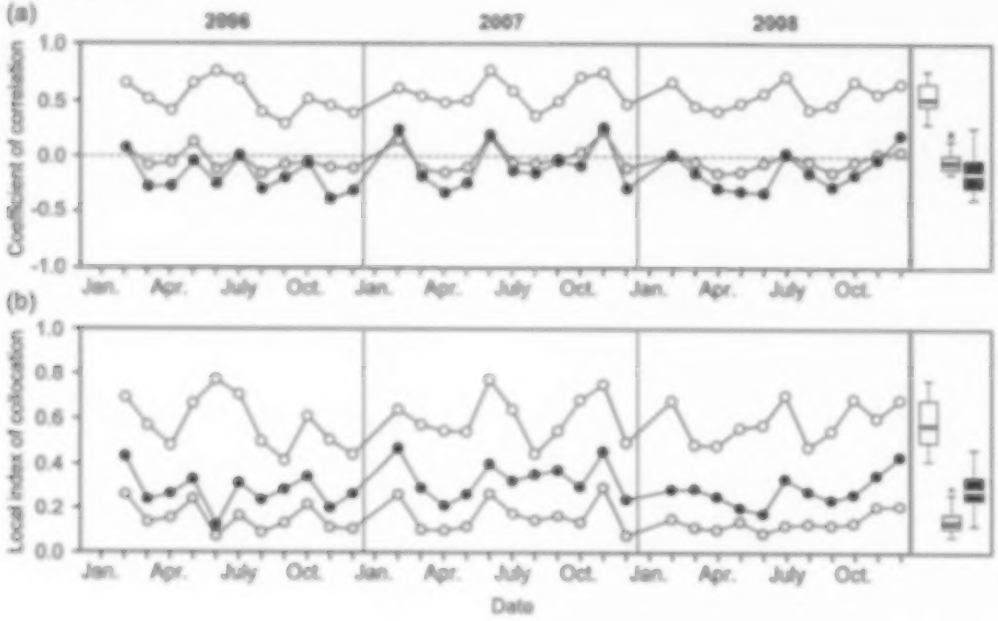


Table 1. Average values of the summary statistics for each activity.

(*a*) Monovariate statistics.

	Fishing	Tracking	Cruising
Time spent (h)	867	1861	1058
Surface of occupancy (km^2)	110 000	405 000	425 000
Gini's index	0.47	0.40	0.18
Nugget (%)	>50	25	50
Spatial range (°)	0.6	1	1

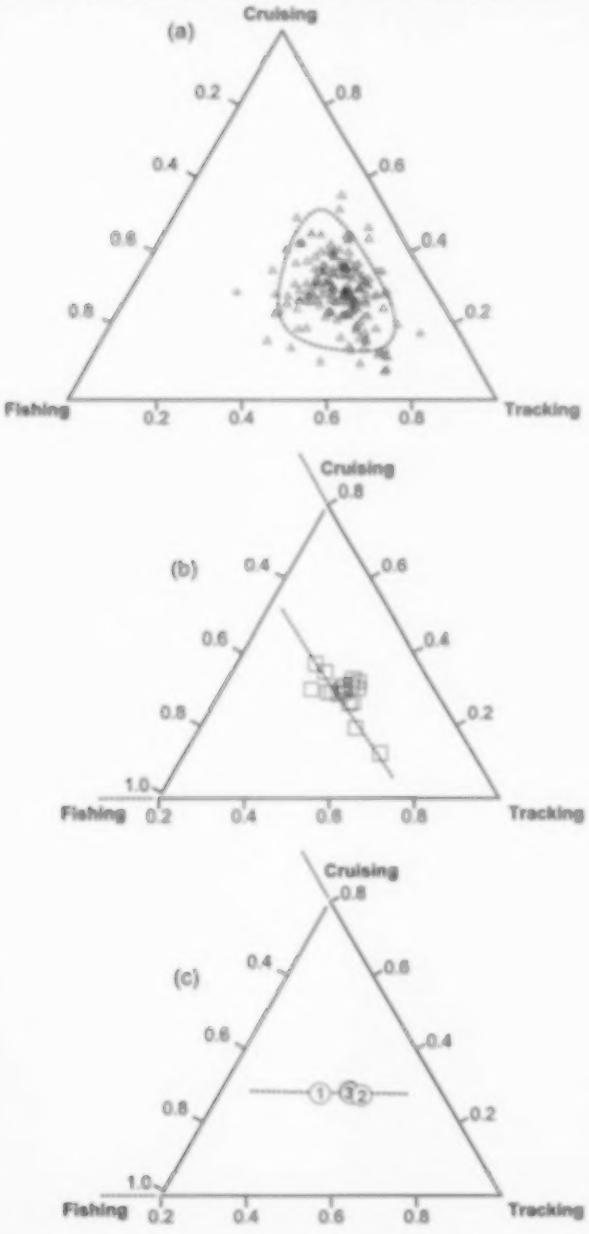
(*b*) Bivariate statistics.

	Fishing × Tracking	Fishing × Cruising	Tracking × Cruising
Coefficient of correlation	0.55	–0.04	–0.13
LIC	0.59	0.15	0.29
Nugget (%)	0	0	0
Spatial range (°)	0.6	—	0.6

Note: Monovariate statistics: time spent (h), surface of occupancy (km^2), Gini's index (no unit), nugget (%), and spatial range (°) of the standardized variograms. Bivariate statistics: coefficient of correlation (no unit), local index of correlation (LIC, no unit), nugget (%), and ranges (°) of the standardized cross-variograms.

al. 2001; Witt and Godley 2007). This may be the reason why most of the analyses of VMS data have only targeted the identification of fishing operations strictly speaking, without considering the vessel activities in between fishing sets (Bastardie et al. 2010; Piet et al. 2007; Mills et al. 2007). A small number of studies were focussed on purse seiners but targeted also the location of the fishing sets (Bertrand et al. 2008; Joo et al. 2011). However, a VMS trajectory, specifically for pelagic fleets, contains much more potential information than the strict location of fishing operations. The searching behaviour of demersal fishing vessels is indeed reduced to a minimum and is not comparable to the larger panel of decisions to which skippers are confronted in pelagic fisheries. Contrary to trawlers, purse seiners, at least when fishing on free-swimming schools, spent the majority of their time searching for rich patch areas and, once found, for fish schools. In the present case, we estimated that the French tropical tuna purse seiners fleet spent 49% of its time searching for schools in areas with clues for tuna aggregations. We also estimated that 25% of the daylight time spent

Fig. 8. Analysis of the fishing strategies. (*a*) Proportions (small triangles) of time spent in fishing, tracking, and cruising activities by vessel and trimester are represented as compositional data (set of values with a closure relationship; Aitchison 1982). The envelope embracing 95% of the observations is represented. Significant effects of a linear model are represented: (*b*) vessel effect (17 modalities; squares) and (*c*) trimester effect (circles, four modalities; Trimester 1 = March–April–May; Trimester 2 = June–July–August; Trimester 3 = September–October–November; Trimester 4 = December–January–February. Vessel effect and trimester effect are orthogonal in the space of the compositions (i.e., parallel to different basis of the triangle); the cross effect was not statistically significant.



at sea (this would be larger if night time were considered, as they allow straight navigation towards new areas or FADs) was dedicated to cruising between rich areas, towards a beacon–FAD, or simply searching for tuna aggregations. However, within the limits of the current available information, we do not know which part of the cruising activity must be associated to change of fishing zones or to a move towards an FAD. We also do not know how to relate this cruising effort to the catches realized under the FADs it allowed to visit. In this sense, the present work is a step towards the definition of an effective fishing effort rather than a formal proposal for a new one.

The majority of the papers analyzing VMS data deal with the classification of VMS data according to the speeds deduced from them and sometimes, but not always, their turning angles. More sophisticated approaches seek for classification based on artificial neural networks (Joo et al. 2011). Recent papers (Vermard et al. 2010; Walker and Bez 2010) also suggested applying hidden Markov models following earlier applications in continental ecology (Jonsen et al. 2005; Morales et al. 2004). One of the advantages of these methods relies on the fact that they take into account sequences of VMS data.

The common weakness of most of these applications is that they were not confronted with field data. The state–space model used in the present study to estimate vessel activity all along the vessel track, step by step, has been validated on the subset of VMS data for which concomitant observers' data were available (Walker and Bez 2010). This was indeed central in our work. From an academic perspective, the fact that the model worked properly (i.e., provided results) and honoured relevant statistical criteria (e.g., mixing and convergence of MCMC chains) would have been considered sufficient. A validation step was necessary when dealing with finalized objective like fisheries management. The misdetection rate was about 10% at the step level. This rate diminished to 3% of under-detection and 15% of over-detection when dealing with fishing sets. Again, case studies where field truth allowed qualifying the model outputs are rare in the literature. Working on another fleet (Peruvian anchovy purse seiners) with another method (artificial neural networks), Joo et al. (2011) got 76% of true positives. Compared with this, the reasonably low level of misdetection we observed makes it possible to use VMS data to infer information on the vessel activity and on fishing effort in an operational perspective.

Fishing effort for the French purse seiners in the Indian Ocean

Wrapping up results

The summary statistics brought several pieces of information about the relative spatial distributions of the vessels' activities at the fleet level. The first three statistics, namely the spatial extensions, the total amounts of time allocated to each type of activity, and the Gini's index, were not spatial statistics strictly speaking, as they were not sensitive to the geolocation of the data. For each statistic, two activities got similar values, but they were different from one statistic to the other, reinforcing the fact that the three statistics formed a complementary set.

Comparing fishing and tracking, we observed that a given proportion of their total allocated times were recovered within the same proportion of area (same Gini's indices) even though the total allocated times and the total areas were strongly different. Tracking and cruising covered the same geographic area, with, overall, twice less time devoted to cruising, making mean density (i.e., the time spent by unit surface) twice smaller for cruising than tracking. Relative to this difference in overall quantities, the proportion of space required to recover a given proportion of the total was smaller for tracking than for cruising. Finally, fishing had a geographical imprint four times smaller than cruising, but both methods had the same total amount of time spent. The density was thus in the same proportion.

The spatial covariations (cross-variograms), positive between fishing and tracking and negative between the other two pairs, were strongly consistent over time. The local spatial variabilities present in each activity were not correlated (nugget effects present in each variogram, but not in the cross-variograms).

Spatial hierarchy in the fishing effort components

As expected, fishing and tracking occurred mainly in the same areas, while cruising occurred in separated areas (LIC and coefficient of correlations). The relative intermediate value of the LIC between fishing and tracking can be explained by the pixel size. At this fine spatial resolution ($0.2° \times 0.2°$ square; i.e., at a fixed time), it is difficult to imagine observing two different activities simultaneously. The time window used in this study (1 month) allowed, however, to get both activities in the same pixel. Their Gini's indices were equally large, indicating that fishing and tracking were similarly patchy. Finally, as fishing occurred in a smaller area than tracking, we can postulate that small patches of fishing activity were included in patchy areas of tracking. In the same way, the large values of surface of occupancy and the small values of Gini's index and of LIC observed for cruising proved that cruising spread in large areas outside fishing and tracking patches.

The large nugget effect and the small spatial range observed for fishing indicated that at the pixel scale, this activity could be viewed as the sum of two random processes: one being pure random and the other producing small patches of three pixels. Tracking activity was more structured (small nugget) over larger spatial aggregation. The whole set of summary statistics supported the principle of a hierarchical spatial organization of the three fishing activities with different scales: tiny to small heterogeneous patches of fishing activity included in intermediate tracking areas themselves included in foraging (cruising) area of the fleet.

Fishing strategies

The state–space model developed in this study allowed a detailed inspection of fishing strategies. Given the spatio-temporal window selected for the analysis, a fishing strategy was quantified by the proportion of daytime spent doing each of the three main activities (i.e., fishing–tracking–cruising) at a month scale. In such context, fishing strategies were likely to change from month to month and from vessel to vessel. The colinearity between strata and time of the year was strong and the two explanatory variables highly redundant. The two remaining variables happened to be orthogonal in their potential to explain the variability of the fishing strategies. Fishing strategies, described by triplets of proportions, were explained by the sum of a vessel effect ($R^2 = 66\%$) and a seasonal effect ($R^2 = 56\%$).

Vessels were not different in their proportions of time devoted to fishing. They were, however, discriminated by the way they allocated the remaining time over tracking and cruising activities. In the literature, one generally opposes obstinate predators (large percentage of tracking) to other ones capable of innovation (large proportions of cruising to survey wide and distant areas). Our analysis indicated that for the French purse seiner fleet, vessels could be discriminated by their more or less innovative versus obstinate nature. However, it is unclear how to relate this aspect with a fishing mode specialization. Considering the cost of a modern purse seiner, locating and exploiting the ephemeral patches of tuna schools with efficacy is a necessity to economically sustain the fleet (Gaertner et al. 1999). In such a context, and because the fact that (*i*) the price of large yellowfin is higher than the price of juveniles of the three major tuna species caught in the Indian Ocean and (*ii*) that large yellowfin are mainly caught in free schools while juveniles are caught under FADs, it makes sense to assume that the trajectory of a vessel targeting preferentially high-value free schools depicts predominantly tracking. However, in reality, even when targeting and tracking free schools, a skipper may decide to move to a far away potential rich area. Conversely, large amounts of drifting floating objects may be found in areas of convergence by chance without the need to locate and to travel to its own FADs. The only observed difference in behaviour between both strategies is that skippers generally prefer exploring fishing grounds alone when they decide to seed or visiting their FADs (Ariz et al. 2002).

Although the area effect was not significant to explain differences in the fishing strategies, the quarters reflected the periodicity of the fleet dynamics. This is a characteristic of the French purse seiner fleet, which follows the tuna migrations and visit, successively, strata 4, 2, 1, and 3. The largest proportion of time devoted to fishing, as opposed to tracking, was observed in May (first quarter in our analysis) when the fleet, and the tuna, seat in the Mozambique Channel, a less opened sea than north off Madagascar, where the fleet spends the rest of the year. This is also when and where tuna habitat is the most dynamic, with strong and sharp gyres moving southwards, generating clear and active fronts (Schouten et al. 2003).

Compared with the information reported in logbooks, in which only one record per day is reported, VMS data opened the possibility (*i*) to georeference multiple fishing events by day and (*ii*) to qualify and quantify the time spent at sea all along a vessel's trip. Fishing effort is thus no longer reduced to a simple number of days at sea or a raw searching time. VMS data associated with an adequate model to analyze them and data to calibrate their performance provided a way to study and quantify the spatial fleet dynamic of the exploitation.

The behavior of a vessel before a fishing operation is a clue for the fishing mode selected and consequently the fish size category targeted. Sets on free-swimming schools are likely to be performed after a tracking phase. On the con-

trary, sets on FADs are likely to be performed after a cruising phase. Still, the automatic discrimination of the type of set realized was not operational at the resolution used in this study, and additional information (e.g., day time of the setting (Harley et al. 2009; Fonteneau et al. 2010), proportion of artificial/natural objects by spatio-temporal strata (Fauvel et al. 2009), etc.) is required. Furthermore, some improvements are expected to be possible when increasing the frequency of VMS acquisition and (or) by the use of logbooks as the quality of the set (i.e., free versus associated schools) is a compulsory piece of information that skippers must register.

A fleet is not a simple addition of vessels. Competitive and cooperative interactions between skippers occur at various spatio-temporal scales. At one extreme, the security rules settled in 2010 to counter the development of piracy in the Indian Ocean force vessel to operate by pairs. This imposed and strong cooperation has severe consequence in terms of effective fishing effort, which should be considered carefully.

Finally, from a bio-logging perspective, it is likely that the vessel behavior could be used directly as an index of abundance without going through the rigorous definition of an effective fishing effort or a CPUE. As a matter of fact, purse seiner fisheries are such that irrespective of the fishing strategies followed by the skippers that lead them to survey space in different ways, the choices of the skipper to fish, versus not to fish, are very good clues for the presence, versus the absence, of fish. In this respect, the behavior of fishermen at sea is considered to have the same informative power about their prey as birds show for their prey (Boyd and Murray 2001; Tremblay et al. 2009). The direct use of the present work to correct the traditional CPUEs could have been an issue. However, we do not know the relative importance one should give to each component of the fishing activity when computing the effective fishing effort. How many times is searching activity more important than cruising in measuring effective fishing effort? Nobody knows. By the way these components are correlated (their sum being the nominal fishing effort), it is likely that their use in a corrected CPUE would not drastically change the results. More importantly, no field truth will ever be available to tune such analyses, which makes useless any comparison between traditional and new CPUEs, as we would then lack objective means to evaluate their difference. Fishermen, and especially seiners, have top predator-like behaviours (Bertrand et al. 2007). We thus believe that the next challenge will be to use the behaviours of the vessels to directly infer the level of the biomass of their prey (in gross terms) without having to compute any catch ratio.

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Effects of size and diet on stable hydrogen isotope values (δD) in fish: implications for tracing origins of individuals and their food sources

David X. Soto, Leonard I. Wassenaar, Keith A. Hobson, and Jordi Catalan

Abstract: Recent studies suggest that stable hydrogen isotope ratios (δD) of fish can be used to track their watershed origin and the relative contributions of source material. Both applications assume that there is no metabolic or trophic effect on tissue δD. We studied the local variation of δD values in four fish species of contrasting size and feeding habits in an isotopically homogenous reservoir (Flix, Spain). Other isotopic values ($\delta^{15}N$, $\delta^{13}C$) and trace metal content were measured as indicators of trophic and bioaccumulation patterns. In addition, isotopic values (δD, $\delta^{15}N$, $\delta^{13}C$) of other food web components were measured for comparison. Muscle δD values differed greatly among fish species: European catfish, *Silurus glanis* (–131‰); common carp, *Cyprinus carpio* (–141‰); rudd, *Scardinius erythrophthalmus* (–158‰); and roach, *Rutilus rutilus* (–163‰). The influence of fish size and trophic position affected the fish δD values. Possible mechanisms that drive δD variation among fish might be a metabolic effect and (or) the compounding effect of ambient water δD on exchangeable H at each trophic stage. Our findings showed that size and feeding habits are factors that should be controlled when tracing origins of fish or their dependence on nutrient inputs using δD measurements.

Résumé : Des études récentes indiquent que les rapports d'isotopes stables d'hydrogène (δD) des poissons peuvent servir à retracer leur bassin versant d'origine et les contributions relatives des matériaux sources. Ces deux utilisations présupposent qu'il n'y a pas d'effet métabolique ou trophique sur le δD des tissus. Nous avons étudié la variation locale des valeurs de δD chez quatre espèces de poissons de tailles et d'habitudes alimentaires très différentes dans un réservoir homogène par ses isotopes (Flix, Espagne). Nous avons déterminé les autres valeurs isotopiques ($\delta^{15}N$, $\delta^{13}C$) et les concentrations des métaux en trace comme indicateurs des patrons trophiques et des patrons de bioconcentration. Nous avons, de plus, mesuré les valeurs isotopiques (δD, $\delta^{15}N$, $\delta^{13}C$) des autres composantes du réseau alimentaire pour établir des comparaisons. Les valeurs de δD du muscle varient considérablement d'une espèce de poisson à une autre : le silure glane, *Silurus glanis* (–131 ‰), la carpe commune, *Cyprinus carpio* (–141 ‰), le rotengle, *Scardinius erythrophthalmus* (–158 ‰) et le gardon, *Rutilus rutilus* (–163 ‰). La taille du poisson et sa position trophique affectent les valeurs de δD du poisson. Les mécanismes explicatifs possibles de la variation de δD chez les poissons pourraient inclure un effet métabolique et (ou) l'effet cumulatif du δD de l'eau ambiante sur l'H échangeable à chaque stade trophique. Nos résultats démontrent qu'il faut tenir compte de la taille et des habitudes alimentaires pour retracer à l'aide de mesures de δD les origines des poissons ou leur dépendance des apports de nutriments.

[Traduit par la Rédaction]

Introduction

Stable isotopes are currently broadly applied in ecological studies. Measurements of stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) are used as indicators of trophic level and dietary sources in aquatic food webs (Peterson and Fry 1987; Hesslein et al. 1991; France 1995). Stable isotopes of hydrogen (δD) have been used to track animal migration (Hobson and Wassenaar 2008) and movements of humans (O'Brien and Wooller 2007; Ehleringer et al. 2008) among isotopically distinct regions. It has been suggested that δD values of fish and invertebrate tissues reflect those of ambient water and can therefore be used to infer the watershed origin of fish as they should reflect the main host stream or lake in which the tissue developed (Whitledge et al. 2006, 2007; Myers et al. 2011). The use of δD for distinguishing among allochthonous and autochthonous energy inputs to aquatic ecosystems has also been proposed (Doucett et al. 2007). Both applications assume negligible trophic effects on hydrogen isotopes. However, patterns of δD related to trophic levels are suggested by Birchall et al. (2005) and Reynard and Hedges (2008). A few controlled-diet experi-

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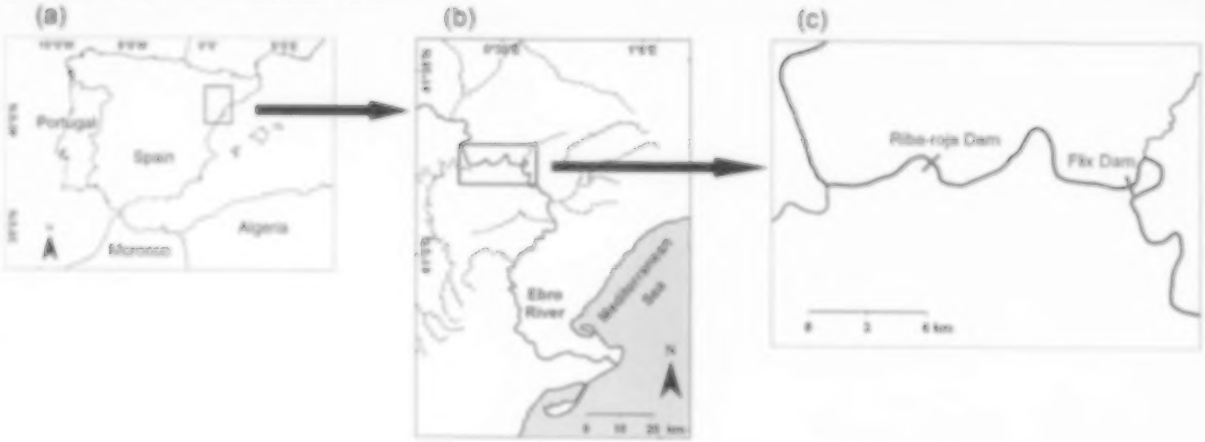
Fig. 1. (*a*) Location of the study area within the Iberian Peninsula, (*b*) location of the Flix reservoir within the lower Ebro River basin, and (*c*) a detailed map of the marked area of (*b*).



ments on terrestrial insects and aquatic organisms suggested that δD trophic enrichment does not occur (Hobson et al. 1999*b*; Solomon et al. 2009). These experimental studies suggest that tissue δD values are influenced by both food and environmental water. Further studies are required to fully understand the mechanisms and controlling factors that determine variation in δD values as these will determine to a large degree how effectively this isotope can be used to trace origins of fish and the nutrients that they use.

Part of the current uncertainty about δD measurements may be due to inconsistency among laboratory methods used for δD measurements. Some authors remove lipids prior to δD analysis (e.g., Jardine et al. 2009; this study), but others do not (e.g., Doucett et al. 2007). Lipids are greatly depleted in deuterium and, depending on lipid content, can significantly impact whole-tissue δD measurements. The deuterium content of lipids is much lower than that of proteinaceous tissues in general (60‰ lower than muscle tissue; Hobson et al. 1999*a*). Differences between non-lipid- and lipid-extracted values in fish muscle can therefore range up to 57‰ (mean, 10.5‰; $n = 84$; L.I. Wassenaar and K.A. Hobson, unpublished freshwater fish data from Lake Winnipeg, Canada). Large δD differences between lipids and water are observed in different types of organisms (Sternberg 1988; Zhang et al. 2009). Moreover, δD measurements need to be conducted using appropriate reference materials that match closely with sample matrix and the fraction of exchangeable hydrogen of the samples of interest (Chesson et al. 2009).

In the current study, we assessed the δD variation in lipid-free muscle within a single fish community in which the hydrogen isotopic composition of ambient water was relatively constant. Our aim was to evaluate to what extent local inter- and intra-specific variability could undermine the application of fish δD as a tracer of watershed origin or their food sources and, eventually, which factors should be taken into consideration to avoid misinterpretations. We investigated δD patterns in coexisting fish populations of four species of contrasting size and feeding habits: European catfish, *Silurus glanis*; common carp, *Cyprinus carpio*; roach, *Rutilus rutilus*; and rudd, *Scardinius erythrophthalmus*. To avoid the potential confounding effect of seasonal and spatial hydrogen isotopic variation in ambient river water and species movement, we performed the study in a reservoir of the Ebro River in which (*i*) the influence of environmental water δD on these fish tissues was the same for all species, (*ii*) the isotopic composition of the river water was known to be homogenous due to storage in upstream large reservoirs, and (*iii*) fish migrations were constrained by up- and down-stream dams. Thus, any differences in tissue δD values should be due to local processes related to fish diet and (or) physiology.

To complement our approach, we measured other tracers: stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in all fish and food sources as trophic indicators. We also determined concentrations of Hg and As in fish as additional indicators of bioaccumulation and exchange related to diet and physiological constraints. Hg and As tend to show opposite behavior as bioaccumulation tracers with increasing trophic level (Cabana and Rasmussen 1994; Kuroiwa et al. 1994; Suhendrayatna et al. 2002). Relationships between Hg concentrations and size can be attributed to fish growth efficiency within a population (Trudel and Rasmussen 2006).

Our approach allowed us to identify the factors that influence δD values in aquatic consumers by controlling for potential movements and to assess the applicability of the δD approach to tracing watershed origins or food sources.

Materials and methods

Study area

The Ebro River is located in the northeastern Iberian Peninsula (southwest Europe). Our study was carried out in the Flix reservoir, located within the lower Ebro River basin (Fig. 1). The Flix reservoir is relatively small (11×10^6 m^3), has a water residence time of 0.3 days (Navarro et al. 2006), and is preceded by the Mequinenza and Riba-roja reservoirs, which have large storage capacities of 1534×10^6 and 207×10^6 m^3, respectively. The maximum depth of the Flix reservoir is <10 m; therefore, the photic zone generally extends to the bottom, resulting in high macrophyte and benthic algal production. The Riba-roja reservoir is located 13 km upstream of the Flix dam. As no fish ladders are present at either dam, fish populations in the Flix reservoir are effectively

isolated from populations above and below the reservoir, thereby preventing sampling of migrant individuals.

Available data indicate that the Ebro River has low spatial variability in water δD. During the summer of 2008, water δD values in the lower basin were –64.6‰ ± 1.2‰ (n = 4), and during the period 2002–2003, δD values throughout the Ebro River watershed were –56‰ ± 6.1‰ (n = 14) (Confederación Hidrográfica del Ebro – Centro de Estudios y Experimentación de Obras Públicas 2003). This low spatial and seasonal isotopic variation results from storage and mixing in upstream large reservoirs and is much lower than the variation observed in the reservoir fish populations in our study (see below). Generally, seasonal variation can be larger in streams where the main source of water is precipitation (e.g., reaches of first order) than in those where it is groundwater. Nielson and Bowen (2010) report a δD variability range of 15‰ from spatially and seasonal distributed values in Great Salt Lake. Data on δD water in Weser River, Germany, show a standard deviation (SD) of <3‰ and ranges of <15‰ in most locations during a 5-year sampling (Koeniger et al. 2009). Therefore, we concluded that the δD variability in Ebro River is also low.

Sampling and sample treatment

Fish sampling was carried out in July 2006, but some (n = 11) catfish were captured in February 2006. Fish were captured in the littoral zone by daylight boat electrofishing (Carol et al. 2006) and were kept alive in oxygenated tanks until processing. Each individual was measured and weighed. Muscle tissue of 20 catfish (*Silurus glanis*), 30 carp (*Cyprinus carpio*), 20 rudd (*Scardinus erythrophthalmus*), and six roach (*Rutilus rutilus*) individuals was dissected and analyzed. Potential food sources of those fish species were also collected. Three sources of autochthonous organic matter were sampled: epilithic biofilms (collected using a soft brush), submerged macrophytes (hand collected), and seston (reservoir water sampled using Niskin bottles at 2–3 m depth and filtered through glass fiber filters). Zooplankton was sampled by means of horizontal transects at a 1 m depth using a plankton net with 200 µm mesh; small macroinvertebrates (i.e., Odonata, Gasteropoda, Coleoptera, etc.) were sampled by disturbing the littoral zone over common reed (*Phragmites australis*) and submerged macrophytes; zebra mussels (*Dreissena polymorpha*) were removed from stones or rizhomes of common reed; crayfish (*Procamburus clarkii*) were sampled using an elastic net overnight; and small fish (<70 mm) of two different species (*Scardinius erythrophthalmus*, n = 12; *Gambusia holbrooki*, n = 20) were sampled by electrofishing and processed whole, in contrast to large fish, which were muscle-subsampled. All samples were frozen at –20 °C, freeze-dried, and then homogenized.

Stable isotope analyses

For $\delta^{15}N$ and $\delta^{13}C$ analyses, samples were weighed into tin capsules and analysed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope-ratio mass spectrometer using standard techniques. Samples for δD measurements were lipid-extracted by soaking and multiple rinses in a solution of 2:1 (v/v) chloroform–methanol and weighed into silver capsules. We used the comparative equilibration method to determine the nonexchangeable hydrogen isotopic composition (Wassenaar and Hobson 2000, 2003) by using three laboratory fish muscle standards (Florida garr (FLG), lake trout (LAT), and Atlantic salmon (ATS)) that were previously calibrated offline for their nonexchangeable δD values (fraction of H exchangeable for fish muscle is 18.5% ± 2.1%; L.I. Wassenaar, Environment Canada, unpublished data). Measurements of δD of samples and standards were performed on H_2 derived from 1350 °C flash pyrolysis and continuous-flow isotope-ratio mass spectrometry (CF-IRMS). Pure H_2 was used as the sample analysis gas and the isotopic reference gas. A Hekatech high-temperature oxygen (HTO) analyzer was used to automatically pyrolyse muscle samples to a single pulse of H_2 gas (and N_2 and CO gas). The resolved H_2 sample pulse was then introduced to the isotope-ratio mass spectrometer (Micromass Isoprime (Manchester, England) with electrostatic analyzer) via an open-split capillary (Wassenaar and Hobson 2006). Water isotope samples were analyzed using an OA-ICOS water isotope analyzer (Los Gatos model 908-0008) coupled to a CTC LC-PAL liquid autosampler for D/H ratios measurements of H_2O (Lis et al. 2008).

All isotopic results are expressed in the typical delta notation, in units of per mil (‰). Nitrogen and carbon isotopic analyses were reported relative to Air and PDB standards, respectively. Hydrogen isotopic analyses were performed at Environment Canada in Saskatoon (Saskatchewan, Canada) and normalized to Vienna Standard Mean Ocean Water (VSMOW). Repeated analyses of isotope laboratory standards yielded an analytical precision better than ±0.2‰ for nitrogen, ±0.3‰ for carbon, and ±2.0‰ and ±0.3‰ (Lis et al. 2008) for hydrogen in tissue and water samples, respectively.

Trace metal analyses

Each fish muscle sample (~70 mg) was digested in 2 mL concentrated HNO_3 (65% Merck Suprapur) and 1 mL H_2O_2 (30% Merck Suprapur) in closed Teflon vessels for two periods of 16 h at 95 °C. After digestion, samples were diluted with deionized water (30 mL) for trace element analysis. A triplicate of blanks was run with each batch of samples.

Analyses of Hg and As were determined by inductively coupled plasma mass-spectrometry (ICP-MS). Determination of metals was performed with a Perkin-Elmer Elan 6000 ICP-MS. Certified ICP standards were used in the calibration of standard curves. Analytical accuracy was checked by a standard reference material (DORM-2, National Research Council of Canada). Accuracy (n = 18) was ±10% for Hg and As. The trace metal concentrations were expressed in dry mass.

Numerical analyses

Trophic position of food web components were estimated by the equation below (Post 2002). We adjusted the estimation to the isotopic baseline of primary consumers using zebra mussels as the pelagic food web reference and snails as the littoral zone reference. We assumed no trophic fractionation in carbon isotopes.

$$\text{TP} = 2 + \frac{\delta^{15}N_{\text{consumer}} - [\delta^{15}N_{\text{snail}}\,\alpha + \delta^{15}N_{\text{mussel}}\,(1-\alpha)]}{\Delta\delta^{15}N} \quad (1)$$

where TP is trophic position, $\delta^{15}N_{\text{consumer}}$ is the $\delta^{15}N$ value of the consumer, $\delta^{15}N_{\text{snail}}$ and $\delta^{15}N_{\text{mussel}}$ are the mean $\delta^{15}N$ va-

lues of snails and zebra mussels, respectively, $\Delta\delta^{15}N$ is the mean trophic discrimination factor for $\delta^{15}N$ (4.8‰ for rudd, the herbivorous fish species (Mill et al. 2007), and 3.4‰ for the rest of aquatic consumers (Post 2002)), and α is the proportion of nutrients in the consumer derived from the base of littoral food web that is estimated according to

$$(2) \qquad \alpha = (\delta^{13}C_{consumer} - \delta^{13}C_{mussel})/(\delta^{13}C_{snail} - \delta^{13}C_{mussel})$$

where $\delta^{13}C_{consumer}$ is the $\delta^{13}C$ value of the consumer and $\delta^{13}C_{mussel}$ and $\delta^{13}C_{snail}$ are the mean $\delta^{13}C$ values of snails and zebra mussels, respectively. For TP calculations, the values of $\delta^{13}C_{consumer}$ were normalized for the effects of lipids on carbon isotopes using eq. 3 of table 1 of Post et al. (2007) for aquatic animals.

Fish length and the trace metal concentrations were $\log_{10}$-transformed in all statistical analyses. Relationships between δD and the other measured variables were examined by Pearson's correlation coefficients. General linear models (GLM) were used to determine which factors influenced fish δD values. We modeled *size* and *trophic* effects on δD values and Hg and As concentrations for fish to determine the factors driving that δD variability. We selected the models using the Akaike information criterion (AIC), and we usually removed nonsignificant variables ($p > 0.05$). We studied the effect of fish length and trophic information (α, TP, and their interaction). Then, because colinearity between length and trophic effects occurs, the residuals of the model between length and trophic information were used to show the effect of size excluding trophic effects.

Results

Patterns of δD, $\delta^{15}N$, and $\delta^{13}C$ values throughout the food web, fish, and their potential food sources differed markedly (Fig. 2). The range of δD values for all fish muscle spanned 49‰ (−169‰ to −118‰). A comparison of the four fish species revealed a decreasing order for δD values as follows: catfish (−131‰ ± 8‰, mean ± SD), carp (−141‰ ± 5‰), rudd (−158‰ ± 8‰), and roach (−163‰ ± 5‰). The δD values differed among species, except between rudd and roach (analysis of variance (ANOVA), $F = 70.28$; Bonferroni's test, $p < 0.01$). A large range of δD values (ca. −200‰ to −100‰) was found for the main components of the food web. Among the potential food items for fish, macrophytes and zebra mussel had high δD values, and zooplankton and biofilm had low δD values (Fig. 2). Although $\delta^{15}N$ and $\delta^{13}C$ values showed a large overlap among the four fish species, some differences were significant. Carp had lower $\delta^{15}N$ and $\delta^{13}C$ values than catfish and rudd, and roach had lower $\delta^{13}C$ values than catfish (ANOVA, Bonferroni's test, $p < 0.05$). Our $\delta^{15}N$ and $\delta^{13}C$ measurements were not useful in separating isotopically among species, and we were unable to model fish diet using conventional C and N stable isotope mixing models (e.g., Phillips and Gregg 2003).

There was a high correlation between the δD values of fish and fish length (Table 1; Fig. 3*a*) and Hg concentrations (Table 1; Fig. 3*d*). In addition, trophic indicators (α and TP) were correlated to Hg concentrations positively and to As concentrations negatively (Table 1). Of note was the high correlation between fish length and Hg concentrations.

We statistically modeled the variability in fish δD values to assess the influences of size and trophic effects. To avoid negative values, instead of α, the variable ($\alpha + 1$) was used in the models as a trophic tracer indicating the littoral origin of food sources (see Fig. 3*c*). Firstly, we found a high variation of fish length explained by trophic indicators (ca. 20%; model 1 in Table 2). Therefore, this collinearity was removed using the residuals of this relationship.

Fig. 2. Box plots of the values of (*a*) δD, (*b*) $\delta^{15}N$, and (*c*) $\delta^{13}C$ for each fish species and their potential food sources from the Flix reservoir. The box plots depict the minimum and maximum values (whiskers), the lower and upper quartiles (25th and 75th percentiles), and the median (central line inside the box). Values more than 1.5 IQRs (interquartile range) are labelled as outliers (solid circles with a line). Dotted lines represent the location of each *x*-axis label to help the visualization of the boxes. Solid triangles depict the values of (*a*), (*b*), and (*c*) for macrophyte, biofilm, and seston samples. Macroinv., macroinvertebrates; Z. mussel, zebra mussel; Zoopl., zooplankton; Macroph., macrophytes.

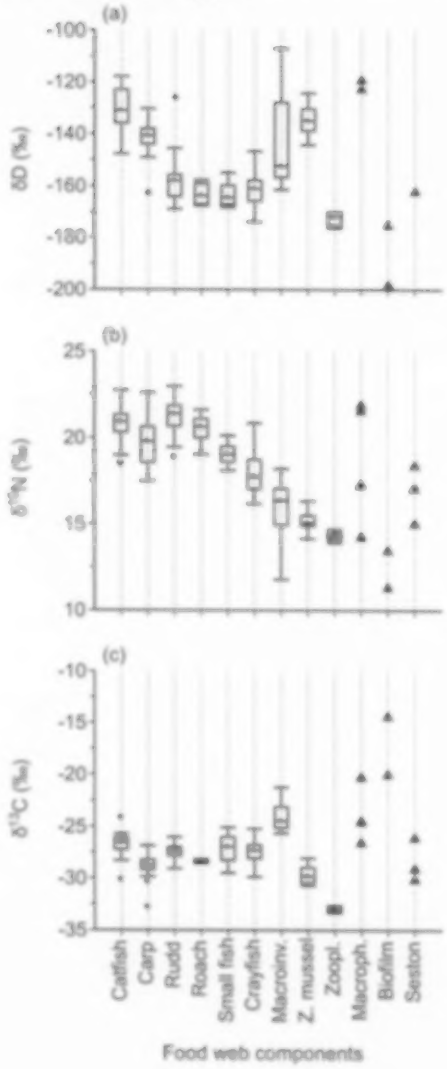
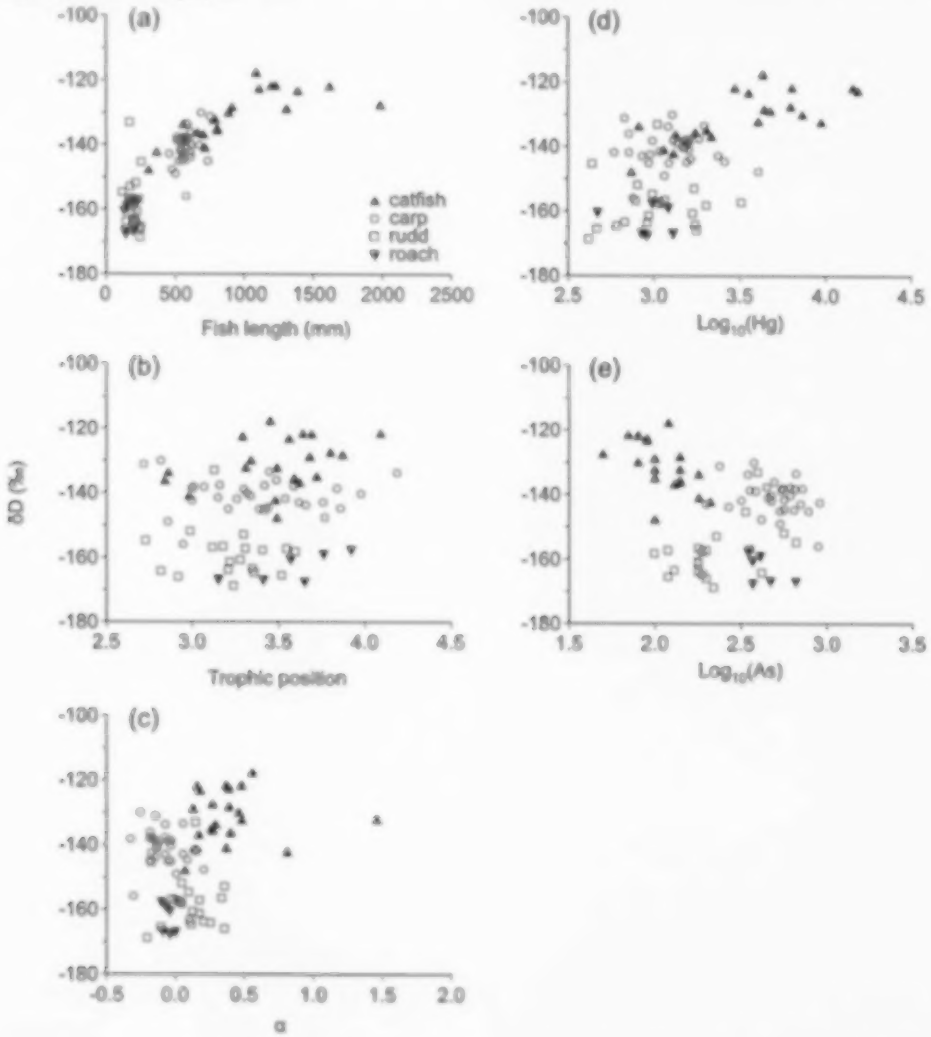


Table 1. Correlation matrix of δD values and their potential predictors (length, α, TP, Hg, and As) for the fish from the Flix reservoir (N = 76).

	δD	Log_{10}(length)	α	TP	Log_{10}(Hg)
Log_{10}(length)	0.88				
α	0.27	0.19			
TP	0.20	0.21	0.04		
Log_{10}(Hg)	0.60	0.58	0.53	0.31	
Log_{10}(As)	–0.26	–0.26	–0.57	–0.28	–0.49

Note: TP, trophic position; α, the proportion of nutrients in the consumer derived from the base of littoral food web.

Fig. 3. Relationship between δD values of fish from the Flix reservoir and potential predictor variables: (*a*) fish length, (*b*) trophic position (TP), (*c*) α, (*d*) log_{10}(Hg), and (*e*) log_{10}(As).



An influence of both trophic position and size effects on fish δD values occurred (models 2–4 in Table 2). Trace metal concentrations (Hg and As) were primarily related to trophic effects, and some influence of size on [Hg] was found (models 5–10 in Table 2). Fish δD values and [Hg] were influenced positively by fish size and the interaction between trophic position (TP) and the variable (α + 1) and were negatively influenced by the parameters TP and (α + 1). In contrast, [As] was only influenced by the interaction between TP and (α + 1).

Table 2. Modeling of size and trophic position effects on δD values and Hg and As concentrations for fish to determine the factors driving the δD variability.

	Effect	Linear model	R^2/adjusted R^2	AIC
Collinearity	TP on length	1. $\text{Log}_{10}(\text{length}) \sim -\text{TP} - (\alpha + 1) + \text{TP}{:}(\alpha + 1)$	0.20/0.17	
δD	Length	2. $\delta\text{D} \sim +\log_{10}(\text{length})$	0.77/0.77	501.98
	TP	3. $\delta\text{D} \sim -\text{TP} - (\alpha + 1) + \text{TP}{:}(\alpha + 1)$	0.22/0.19	599.08
	Size + TP	4. $\delta\text{D} \sim +\text{resid(model 1)} - \text{TP} - (\alpha + 1) + \text{TP}{:}(\alpha + 1)$	0.79/0.77	503.23
Log_{10}(Hg)	Length	5. $\text{Log}_{10}(\text{Hg}) \sim +\log_{10}(\text{length})$	0.33/0.32	23.27
	TP	6. $\text{Log}_{10}(\text{Hg}) \sim -(\alpha + 1) - \text{TP} + \text{TP}{:}(\alpha + 1)$	0.43/0.41	14.91
	Size + TP	7. $\text{Log}_{10}(\text{Hg}) \sim +\text{resid(model 1)} - \text{TP} - (\alpha + 1) + \text{TP}{:}(\alpha + 1)$	0.56/0.54	−2.94
Log_{10}(As)	Length	8. $\text{Log}_{10}(\text{As}) \sim -\log_{10}(\text{length})$	0.07/0.05	43.46
	TP	9. $\text{Log}_{10}(\text{As}) \sim -\text{TP}{:}(\alpha + 1)$	0.39/0.38	10.86
	Size + TP	10. $\text{Log}_{10}(\text{As}) \sim -\text{resid(model 1)}^{a} - \text{TP}{:}(\alpha + 1)$	0.40/0.38	12.38

Note: The studied predictor variables are $\log_{10}$(length), trophic position (TP), $(\alpha + 1)$, and the interaction between trophic position and % littoral origin (TP:$(\alpha + 1)$). Before each predictor variable, the symbol of that influence (+ or −) is depicted. In each case, best models were selected using the AIC values as a measure of the relative goodness of fit of the statistical model (lower AIC values) and removing nonsignificant variables ($p > 0.05$). The coefficients of determination (R^2) and the adjusted value for the number of explanatory terms (adjusted R^2) in each model are shown as the proportion of variability that is accounted for by the statistical model. Because of the collinearity between size and trophic effects, the residuals (resid) of their relationship (model 1) were used to show the effect of size removing the explained variation by trophic effects in next models.

[a]Nonsignificant parameter in the best model.

Discussion

Our results showed that δD in lipid-extracted fish muscle tissue varied considerably among species and within populations in a single location, even when there was low isotopic variation in ambient water. This result warns against uncritically using δD for tracing watershed origins of organisms. For instance, using the regression model of Whitledge et al. (2006), linking fish tissue δD with water δD, the range of tissue values that we found would mistakenly correspond to a range in host water δD of nearly 100‰. Therefore, unless the factors that control tissue δD values, beyond ambient water, are taken into account, serious errors in assignment of fish to watershed origin may result. When tracing watershed origin of organisms, a size and trophic effect should be controlled, for example, by using fish of similar size and feeding habits. Moreover, studies using δD as a tracer of food sources or terrestrial inputs that do not take into account the influence of size and trophic position (TP), as well as the environmental water contribution to tissue H, should be avoided. A good example of the cautious use of δD as a tracer was provided by Cole et al. (2011), who assessed the terrestrial subsidies to pelagic consumers. They used zooplankton taxa from a similar TP that fed on some mixture of algal and terrestrial resources (treated as primary consumers) and a dietary water influence of 15%.

In our study, possible mechanisms that may have caused variation in fish tissue δD include (*i*) trophic deuterium discrimination in protein H, (*ii*) a metabolic effect related to size, and (*iii*) a compounding effect of isotope exchange with ambient water δD at each trophic level (Solomon et al. 2009). We considered each of these mechanisms in more detail below.

Trophic discrimination

Trophic discrimination in δD similar to δ^{15}N did not occur as δ^{15}N values were not related with δD values. Controlled experimental tests of δD isotopic discrimination under laboratory conditions have been made for monarch butterflies (*Danaus plexippus*; Hobson et al. 1999*b*) and aquatic consumers (Solomon et al. 2009). In both cases, δD showed negligible trophic enrichment. Trophic deuterium enrichment in carnivores is suggested by Birchall et al. (2005) and Reynard and Hedges (2008) based on bone collagen studies. They suggested that δD could be a trophic level indicator similar to δ^{15}N. However, in other cases, trophic level patterns are attributed to other mechanisms. Solomon et al. (2009) showed that consumers at higher trophic levels within distinct components of lake food webs such as zooplankton, zoobenthos, and fish also have higher δD values and provided an explanation based on a compounding effect of water δD at each trophic level (see below). Doucett et al. (2007) also found higher δD values in top-level predators (fish) than in lower trophic levels (macroinvertebrates) and attributed such difference to δD variation in diet items and heterogeneity among basal trophic levels.

Metabolic effect

Metabolic effects related to fish size may have contributed to the variance that we measured for fish muscle δD. An important influence of fish size on both δD values and [Hg] was observed, and [Hg] was also correlated with δD values. Tissue δD values could increase with time or age (which is allometrically related to size), similar to that observed for fish δ^{15}N (Overman and Parrish 2001). This mechanism could be due to the formation of new proteins by catabolic amino acids and the additive influence of H isotope exchange from environmental water into these amino acids. Therefore, the age of protein molecules is expected to play an important role. Catfish was the only species with a significant and positive correlation between δD and [Hg] or size, possibly because of their large size range. Whitledge et al. (2006) assessed the influence of fish length on standardized differences between mean ambient water δD and fish δD values and found no correlation. Therefore, further investigation is required to determine under which circumstances size could significantly influence differences in fish δD values.

Compounding effect

The compounding effect of ambient water δD with trophic level is a mechanism that may show an apparent trophic ef-

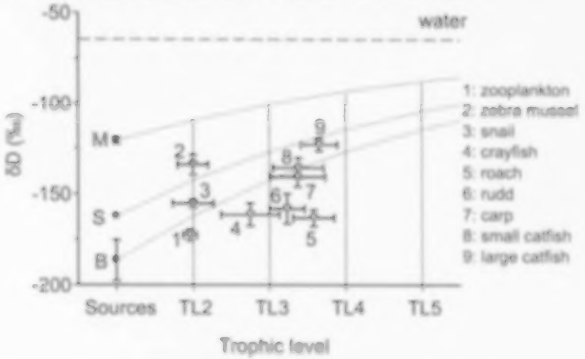
fect related to real dietary trophic position and food source. At each trophic level, there is an opportunity for the additive influence of H isotope exchange between consumer tissue and ambient water in addition to that which has already occurred in the diet (Solomon et al. 2009). Previous studies show that the contribution of ambient water H to consumer tissue in the form of exchangeable H varies widely among organisms and tissue type (Hobson et al. 1999*a*; Solomon et al. 2009; Wang et al. 2009). Hydrogen isotopes in fish body water are highly correlated with environmental water, and H transfer to free amino acids ultimately incorporates the ambient water isotopic influence into fish protein (Gasier et al. 2009). Thus, Solomon et al. (2009) suggest that trophic compounding, or accumulation of the effect of environmental water H along trophic levels, can also contribute to consumer tissue in aquatic food chains. They propose a model incorporating the effect of H isotopic exchange between water and ingested food at each trophic level. Their model predicts, assuming 20% exchangeable H in all tissues and prey items, that H in consumer fish tissues includes the 20% of H obtained from ambient water plus the 20% that their prey obtained from water, and so on. If this proposed model proves valid, fish δD should be correlated with the total contribution of environmental water to tissue H, which, in turn, depends on the trophic level of a consumer and the δD value of the ambient water and food source.

The compounding effect may be another possible mechanism contributing to the variation in fish δD that we observed along with the size effects that we described. We did not find a direct correlation between δD values and trophic position or α. However, the combination of TP and α (plus their interaction) had an influence on fish δD values. The relationship between δD values and trace metals (Hg, positively, and As, negatively) also indicated some trophic and size effects, as these trace metals can be biomagnified and biodiminished along the food chain (Cabana and Rasmussen 1994; Chen and Folt 2000).

To investigate the potential influence of the compounding effect, we modeled the theoretical δD values expected in linear food chains using our measured sources of primary production in the Flix reservoir (macrophytes, seston, and biofilm). We assumed that each consumer ate only from the previous lower trophic level and that the influence of environmental water to tissue H was constant (20%) for all consumers (Fig. 4). The theoretical values of δD for consumer tissues in each linear food chain (i.e., those based entirely on either biofilm, seston, or macrophytes) were calculated using eq. 3 of Solomon et al. (2009) and the water δD value of the reservoir. We placed our measured δD values of consumers using estimates of trophic position on this modeled depiction (Fig. 4). We separated large catfish (>100 cm), which feed mainly on crayfish and fish and so had the highest trophic position, from small catfish (<100 cm), which feed on crayfish, other invertebrates, and plant material (Carol et al. 2009).

The position of the two size classes of catfish (Fig. 4) corresponded well with their assumed diet. Among fish, we expected catfish to occupy the highest trophic position and rudd the lowest. However, our calculated trophic position of rudd was higher than expected. We suspect that this result was driven by consumption of macrophytes by rudd because

Fig. 4. Assessment of H compounding effect as a potential mechanism explaining δD variation: the δD values of tissue may vary depending on the trophic level of the consumer (TL) and the source of energy (Sources). Assuming a linear food chain in which each consumer eats only from the previous lower trophic level and that the compounding influence of environmental water is constant (20%), estimates of δD trophic trajectories starting at three dietary sources (macrophytes, M; seston, S; and biofilm, B) were calculated. Based on measured δD and trophic position estimates from $\delta^{15}N$ and $\delta^{13}C$, positions in the diagram of fish and other important dietary components are plotted. Sources and food web components are represented in the plot by solid circles and open inverted triangles, respectively. Reservoir water is represented by a broken horizontal line.



their diet is mainly based on detritus and plant material (García-Berthou and Moreno-Amich 2000). Macrophytes had relatively high $\delta^{15}N$ values in the reservoir and lower diet protein quality than other food items (i.e., fish), and our estimates of trophic position for rudd could therefore be equivocal (Fig. 4). Similarly, for carp and roach, we expected that they would occupy similar positions in Fig. 4 as they have similar feeding habits, but they had different δD values. Carp is a benthivorous species and likely fed on zebra mussel, macroinvertebrates, and biofilm, whereas roach likely fed more on zooplankton (García-Berthou 1999, 2001). Therefore, the different δD values between them were likely due to their size differences.

Our statistical model of the trophic influence on δD values was consistent with the theoretical δD modeling using trophic compounding (Fig. 4). The values of α were negatively related to δD values, and we found that the littoral primary consumers had more negative values than the more pelagic zebra mussel (Fig. 4). The interaction between trophic position and α was positively related to δD values, and the values of this interaction increased from the bottom left to the top right of Fig. 4. In contrast, trophic position had a negative influence in the model. Regarding the compounding effect isotope model shown (Fig. 4), the influence of water incorporation at each trophic step caused the overall trophic chain to move towards the isotopic composition of the ambient water, but the slope of the hydrogen isotopic enrichment at each trophic level diminished accordingly.

In conclusion, our data showed that variation of fish δD values, even within a single well-mixed reservoir, can be considerable, and such isotopic variation needs to be considered in studies using δD to infer watershed origins of fish and food sources. We concluded that the mechanisms that likely

drive this variation were related to size and compounding effects of ambient water δD. Therefore, fish size and their diet should be taken into account to compare populations from different locations when tracing watershed origins of fish using δD measurements. The use of δD measurements may eventually complement the more traditional use of $\delta^{13}C$ and $\delta^{15}N$ for evaluating trophic and food web structure (Jardine et al. 2009; Solomon et al. 2009; Finlay et al. 2010), but the fundamental mechanisms driving the local hydrogen isotopic variation in food webs and the uncertainties related to lipid content of each food web component remains poorly understood. More controlled experimental studies deciphering and quantifying the mechanisms and effects are needed before a more general application of δD measurements in ecological aquatic studies can be undertaken.

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Quantifying importance of marine prey in the diets of two partially anadromous fishes

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Abstract: The importance of marine prey sources for partially anadromous fishes has received little study and is complicated by considerable plasticity in life history. We determined proportional contributions of marine, freshwater benthic, and freshwater pelagic prey to anadromous and resident Arctic char (*Salvelinus alpinus*) and lake trout (*Salvelinus namaycush*) from several partially anadromous populations in the Canadian Arctic. Bayesian mixing models (MixSIR) applied to $\delta^{34}S$ and $\delta^{13}C$ ratios in lake-specific models indicated that marine prey accounted for 90%–91% of anadromous Arctic char diet and 60%–66% of anadromous lake trout diet. When these estimates were combined with proportion of anadromous individuals in partially anadromous populations, marine food sources accounted for 31%–44% of Arctic char population productivity and 16%–26% of lake trout population productivity. Although future research is needed to refine and quantify variability around these estimates, our results will allow better predictions of the effects of anthropogenic stressors on partially anadromous fishes.

Résumé : On a peu étudié l'importance des sources de proies marines pour les poissons partiellement anadromes et cette étude est compliquée par la très grande plasticité des cycles biologiques. Nous avons mesuré les contributions proportionnelles des proies marines, benthiques d'eau douce et pélagiques d'eau douce chez des ombles chevaliers (*Salvelinus alpinus*) et des touladis (*Salvelinus namaycush*) anadromes et sédentaires dans plusieurs populations partiellement anadromes dans l'Arctique canadien. Des modèles de mélange bayésiens (MixSIR) utilisés sur des rapports $\delta^{34}S$ et $\delta^{13}C$ dans des modèles spécifiques aux lacs indiquent que les proies marines représentent 90–91 % du régime alimentaire des ombles chevaliers anadromes et 60–65 % du régime alimentaire des touladis anadromes. Une fois ces estimations combinées à la proportion d'individus anadromes dans les populations partiellement anadromes, les sources alimentaires marines expliquent 31–44 % de la productivité de la population d'ombles chevaliers et 16–26 % de la productivité de la population de touladis. Bien qu'il faille des recherches supplémentaires pour préciser et mesurer la variabilité de ces estimations, nos résultats permettent de meilleures prédictions des effets des facteurs anthropiques de stress sur les poissons partiellement anadromes.

[Traduit par la Rédaction]

Introduction

Arctic char (*Salvelinus alpinus*) and lake trout (*Salvelinus namaycush*) are of economic, cultural, and subsistence importance in the Canadian Arctic, and their presence in Arctic lakes affects food web structure, contaminant bioaccumulation, and ecology of sympatric freshwater fishes (Van Oostdam et al. 2003; Swanson and Kidd 2010; Swanson et al. 2010*a*). Arctic char and lake trout have very plastic life histories, and in the Canadian Arctic, there are partially anadromous populations of both species (e.g., Johnson 1980; Swanson et al. 2010*b*). In these populations, some individuals remain freshwater residents, whereas others become anadromous after several years of rearing in freshwater. The importance of freshwater and marine food sources to partially anadromous fish populations has received little study and is thus highly uncertain. This uncertainty complicates fisheries and habitat management and makes it difficult to predict how population viability and persistence will be affected by anthropogenic stressors.

Marine migrations are not obligate in partially anadromous fish populations, and individual fish may change their life history tactic (i.e., anadromous or freshwater resident) to maximize fitness if environmental conditions change (e.g., Nordeng 1983; Jonsson and Jonsson 1993). A preliminary study by Swanson and Kidd (2009) found that marine food sources accounted for ~95% of the diet of four anadromous Arctic char captured from one partially anadromous population, but further research is needed to refine this estimate and verify that it is applicable beyond the one studied popu-

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lation. There are currently no estimates of marine vs. freshwater prey use for partially semi-anadromous lake trout that were recently discovered in the Canadian Arctic (Swanson et al. 2010*b*). Lake trout are likely not capable of long-term survival in full-strength seawater (Hiroi and McCormick 2007), and evidence from otolith microchemistry suggests that they either make shorter marine migrations or use lower salinity habitats than Arctic char (Swanson et al. 2010*b*). This may mean that lake trout utlilize marine prey sources to a lesser extent than Arctic char.

Stable isotope mixing models can be used to determine relative contributions of marine and freshwater prey (i.e., sources) to predator diets. Stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotope ratios can be used as tracers because all of these isotope ratios are elevated in marine food sources relative to freshwater food sources (Fry and Sherr 1984; Peterson and Fry 1987). $\delta^{34}S$ and $\delta^{13}C$ are often more useful than $\delta^{15}N$ in mixing models because they do not fractionate appreciably with trophic transfer (e.g., Connolly et al. 2004).

Once prey sources, predators of interest, and isotope tracers have been defined, there are a number of stable isotope mixing models that can be employed. One of the more recent models is MixSIR (Moore and Semmens 2008). This Bayesian model can incorporate prior information and account for uncertainty in isotope ratios and isotope fractionation.

The objective of this study was to determine the relative importance of marine and freshwater prey sources to several populations of partially anadromous Arctic char and lake trout in the Canadian Arctic. MixSIR models were used to determine proportional contributions of three prey sources (marine, freshwater benthic, freshwater pelagic) to diets of anadromous and resident fishes of both species. Using these results and previously published data on proportion of anadromous individuals in partially anadromous populations, marine contributions to population productivity were estimated.

Materials and methods

Field sampling

During the open-water seasons of 2006–2008, anadromous and freshwater-resident (i.e., resident) Arctic char and lake trout were captured in four lakes and outflows located near Hope Bay, Nunavut, Canada (68.1°N, 106.6°W; Fig. 1). Anadromous and resident lake trout were captured in Glenn, Roberts, and Nauyuk lakes, whereas anadromous and resident Arctic char were captured in Hovaktok, Nauyuk, and Roberts lakes. Sampling for anadromous fishes took place in late summer and early fall when fishes were moving upstream, whereas sampling for resident fishes occurred throughout the summer. The target sample size was 20 fish/species/lake (anadromous and resident individuals could not be externally identified in most cases). Fishes were captured using a backpack electrofisher (Type 12, Smith-Root Inc., Vancouver, Washington), sinking gill nets (mesh sizes 1.9 to 8.9 cm stretched mesh), and angling. Because we found that $\delta^{34}S$ ratios differed significantly among lakes (see Results), fish caught in estuaries had to be excluded from further analysis unless their freshwater origin could be independently determined from a tag (for information on tagging studies, see Miramar Hope Bay Ltd. 2005).

Anadromous and resident fishes were differentiated in a previous study using analyses of otolith microchemistry (Swanson et al. 2010*b*). Because isotopic turnover is likely at least one year in these fishes (e.g., Hesslein et al. 1993; MacAvoy et al. 2001) and may be much longer, anadromous fishes that had not completed at least two continuous marine migrations before capture were excluded from further analysis. Captured fishes were measured (to the nearest millimetre) and weighed (to the nearest gram) in the field and dissected for dorsal muscle tissue. Descriptive statistics on fish length, age, and stomach contents are presented in the online supplementary data (Supplemental Tables S1 and S2).[1]

Freshwater food sources (i.e., endmembers in the stable isotope mixing models) were represented by invertebrates. Isopods (*Saduria entomon*) represented a freshwater benthic food source (endmember), whereas zooplankton represented a freshwater pelagic food source (endmember). Isopods and zooplankton were collected from all lakes and from two sites in the marine environment of Melville Sound (Fig. 1). Isopods were collected with kick nets and an Ekman grab in at least two open-water seasons between 2006 and 2008. Between two and 12 replicates were collected from each sampling site. Bulk zooplankton samples were collected in 2008 using horizontal tows with a 153 μm net (0.25 m diameter opening). Three replicates were collected from each lake and from each site in Melville Sound.

Marine food sources were represented by the marine forage fishes capelin (*Mallotus villosus*) and Pacific herring (*Clupea pallasii*). These fishes were noted in several stomachs of anadromous fishes (H. Swanson, unpublished data) and were collected with fyke nets in 2008 from two sites in Melville Sound (Fig. 1). Fishes were measured, weighed, and dissected for dorsal muscle tissue. All samples were frozen within 12 h of collection and processing.

Laboratory analyses

Fish tissue, whole isopods, and bulk zooplankton samples were freeze-dried and ground to a fine powder with a mortar and pestle. When single benthic invertebrates did not provide enough mass for stable isotope analysis, multiple individuals were pooled to form a composite sample. From each lake, two to 12 isopod and zooplankton samples and a minimum of five fish per species – life history type were analyzed for stable C and S isotope ratios. Eight marine forage fishes (four each of Pacific herring and capelin) from Melville Sound were analyzed for stable C and S isotope ratios.

Stable C and S isotope ratios were determined at the University of New Brunswick and Northern Arizona University, respectively, using methods described in Swanson et al. (2010*b*). Isotope ratios are expressed as parts per mil (‰) delta values ($\delta^{13}C$, $\delta^{34}S$) from international standards.

Data analysis

Statistical analyses were performed in SAS (version 9.1.3, SAS Institute Inc. 2002). Alpha (α) was set at 0.05. Before mixing models were run, $\delta^{34}S$ and $\delta^{13}C$ ratios were compared among endmembers and lakes with analyses of variance

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-111.

Fig. 1. Map of the study area in the West Kitikmeot region of Nunavut, Canada. Lake trout (*Salvelinus namaycush*) were collected from Glenn, Roberts, and Nauyuk lakes, and Arctic char (*Salvelinus alpinus*) were collected from Roberts, Hovaktok, and Nauyuk lakes. Stars represent sampling locations in Melville Sound.

(ANOVAs) and *t* tests. Within anadromous fishes, $\delta^{34}S$ and $\delta^{13}C$ ratios were compared between species with a mixed model. Fork length and age were tested as covariates, and lake was a random effect. $\delta^{34}S$ and $\delta^{13}C$ ratios were then compared between anadromous and resident fishes for each lake and species with analyses of covariance (ANCOVA); fork length was the covariate. Relationships between isotope ratios and fish fork length are presented for each species – life history type in the supplementary data.[1]

MixSIR models (Moore and Semmens 2008) were run for anadromous and resident Arctic char and lake trout in each lake, except for Arctic char in Nauyuk Lake (see $\delta^{34}S$ comparisons in Results). Models were run using three endmembers (marine, freshwater benthic, freshwater pelagic) and two isotope tracers ($\delta^{34}S$ and $\delta^{13}C$). Marine food sources were represented by pooled isotope data for capelin and Pacific herring, freshwater benthic food sources were represented by isopods, and freshwater pelagic food sources were represented by zooplankton. Fractionation for both $\delta^{34}S$ and $\delta^{13}C$ was assumed to be +0.4 ‰ per trophic level (McCutchan et al. 2003). Neither stomach contents nor stable isotope ratios indicated that any of the captured Arctic char were cannibalistic. Arctic char and lake trout were assumed to be 1.5 trophic levels above isopods, two trophic levels above zooplankton, and one trophic level above marine forage fishes. Standard deviation for $\delta^{34}S$ and $\delta^{13}C$ fractionation was calculated to be 1.87‰ and 1.26‰ per trophic level, respectively (calculated from McCutchan et al. 2003). Priors were set to be uninformative and 1 000 000 iterations were used for each run. Model diagnostics for MixSIR were acceptable for all runs; more than 1000 posterior draws were used, there were no duplicate draws, the number of unique parameter vectors in the resample was always equal to the number of iterations, and the maximum importance ratio was always less than 0.001 (Semmens and Moore 2008).

Proportional contributions of marine prey were qualitatively compared between fish species and life history types. To estimate marine contributions to population productivity, 95% confidence intervals for proportional contributions of marine prey to anadromous fish diet were multiplied by species-specific proportions of anadromous fishes in each partially anadromous population (calculated from Swanson et al. 2010*b*).

Results

$\delta^{34}S$ and $\delta^{13}C$ ratios

As expected, $\delta^{34}S$ ratios effectively separated freshwater and marine endmembers, whereas $\delta^{13}C$ ratios effectively separated freshwater benthic and pelagic endmembers. There was a minimum separation of 14‰ between marine and freshwater endmember $\delta^{34}S$, and a minimum separation of 7‰ between freshwater benthic and pelagic $\delta^{13}C$ (Table 1). Both of these differences were significant (ANOVA, $F_{[\geq 2,50]} \geq 9.93$, $P \leq 0.0002$). We observed unexpectedly high among-lake variation in freshwater endmember $\delta^{34}S$;

Table 1. Mean ± standard deviation and sample size (N) for $\delta^{34}S$ and $\delta^{13}C$ ratios determined for marine and freshwater endmembers.

	$\delta^{34}S$ (‰)			$\delta^{13}C$ (‰)		
	Freshwater benthic	Freshwater pelagic	Marine	Freshwater benthic	Freshwater pelagic	Marine
Glenn	–8.69±0.72 (6)	–7.62±0.14 (3)	18.02±0.58 (8)	–24.30±2.25 (6)	–32.05±0.04 (3)	–23.62±1.14 (8)
Hovaktok	4.17±0.81 (2)	3.26±0.07 (3)	18.02±0.58 (8)	–24.32±1.63 (5)	–32.03±0.03 (3)	–23.62±1.14 (8)
Nauyuk	–11.11±1.13 (2)	–8.88±0.12 (3)	18.02±0.58 (8)	–22.07±1.35 (6)	–30.10±0.04 (3)	–23.62±1.14 (8)
Roberts	3.21±0.78 (12)	1.85±0.13 (3)	18.02±0.58 (8)	–24.00±1.81 (9)	–32.51±0.12 (3)	–23.62±1.14 (8)

the range was approximately 15‰ (Table 1), and although $\delta^{34}S$ did not differ between freshwater benthic and pelagic endmembers (t test, $t_{[32]} = 0.73$, $P = 0.47$), among-lake differences were significant (ANOVA, $F_{[3,28]} = 897$, $P < 0.0001$). $\delta^{13}C$ ratios in freshwater endmembers did not differ among lakes (ANOVA, $F_{[3,34]} = 2.71$, $P = 0.06$).

Qualitative comparisons of $\delta^{34}S$ between fishes and endmembers revealed that mixing models would likely be successful for all lake–species combinations, with the exception of Arctic char from Nauyuk Lake. In general, $\delta^{34}S$ ratios for anadromous Arctic char were very close to those of marine endmembers, whereas $\delta^{34}S$ ratios for resident Arctic char were very close to those of freshwater endmembers (Fig. 2*a*). In Nauyuk Lake, however, resident Arctic char had $\delta^{34}S$ ratios that were inconsistent with freshwater feeding (Fig. 2*a*). Arctic char from Nauyuk Lake were thus not included in mixing models, and possible reasons for high $\delta^{34}S$ ratios in these resident char are discussed below. Qualitative comparisons also indicated that anadromous lake trout utilized marine prey sources to a lesser extent than anadromous Arctic char (Fig. 2*b*). Results of a mixed model indicated that anadromous Arctic char had significantly higher $\delta^{34}S$ ratios than anadromous lake trout ($F_{[1,40]} = 92.3$, $P < 0.0001$). Neither fork length nor age were significant in this model ($F_{[\geq 1,41]} \leq 0.93$, $P \geq 0.28$). In each lake and for both species, $\delta^{34}S$ ratios were significantly higher in anadromous fishes than in resident fishes (ANCOVAs, $F_{[\geq 1,5]} \geq 52.03$, $P \leq 0.0001$).

As mentioned above, $\delta^{13}C$ ratios did not separate marine and freshwater endmembers as well as $\delta^{34}S$ ratios. Marine endmembers, freshwater benthic endmembers, anadromous Arctic char, and anadromous lake trout had similar and relatively enriched $\delta^{13}C$ (Figs. 2*c*, 2*d*). The freshwater pelagic endmember (zooplankton) had more negative $\delta^{13}C$ ratios, and $\delta^{13}C$ ratios for resident fishes indicated use of both freshwater benthic and pelagic food sources (Figs. 2*c*, 2*d*). Anadromous Arctic char had significantly less negative $\delta^{13}C$ ratios than resident Arctic char in Nauyuk and Roberts lakes (ANCOVAs, $F_{[\geq 1,10]} \geq 4.66$, $P < 0.05$). There was no significant difference in $\delta^{13}C$ between anadromous and resident Arctic char in Hovaktok Lake (ANCOVA, $F_{[1,18]} = 0.06$, $P = 0.81$). In all lakes, anadromous lake trout had significantly less negative $\delta^{13}C$ ratios than resident lake trout (ANCOVAs, $F_{[\geq 1,21]} \geq 9.34$, $P < 0.006$) (Fig. 2*d*). A mixed model indicated that anadromous lake trout had significantly more enriched $\delta^{13}C$ than anadromous Arctic char ($F_{[1,59]} = 41.99$, $P < 0.0001$). Similar to the $\delta^{34}S$ analysis, neither fork length nor age were significant covariates in this model ($F_{[\geq 1,47]} \leq 1.67$, $P \geq 0.2$).

Mixing models

Results from MixSIR models indicated that marine food sources accounted for 90%–91% (median) of anadromous Arctic char diet and 60%–66% of anadromous lake trout diet (Table 2; Fig. 3). The remainder of anadromous lake trout diet was primarily accounted for by freshwater benthic sources; only 1% of anadromous lake trout diet was estimated to originate from freshwater pelagic sources (Table 2; Fig. 3). Of the small proportion of freshwater food sources contributing to anadromous Arctic char diet, benthic sources were more important than pelagic sources (5%–8% vs. 2%–3%, respectively) (Table 2). In three of four lakes, freshwater benthic prey accounted for a larger proportion (66%–73%) of resident fish diet than freshwater pelagic prey (17%–34%), but in Roberts Lake, freshwater pelagic prey were more important (Table 2; Fig. 3).

At the population level, anadromous Arctic char used more marine food sources than anadromous lake trout. We estimated that marine food sources accounted for 31%–44% of the productivity of sampled Arctic char populations and 16%–26% of the productivity of sampled lake trout populations (Table 3).

Discussion

Isotope ratios in endmember taxa

Consistent with the review of Connolly et al. (2004), marine and freshwater food sources were better separated by $\delta^{34}S$ than by $\delta^{13}C$. Using $\delta^{34}S$ as an isotope tracer was more complex than expected, however, because of high among-lake variability in $\delta^{34}S$ ratios. This meant that mixing models had to be run separately for each lake.

$\delta^{34}S$ ratios in freshwater endmembers ranged more than 15‰ among the study lakes, and this was not likely a result of differences in endmember feeding ecology or habitat use. Sulfur does not fractionate appreciably with trophic transfer (e.g., Peterson and Fry 1987), so it is unlikely that observed among-lake variation in $\delta^{34}S$ resulted from differences in endmember trophic level. It is also unlikely that these differences reflected variation in habitat use. Zooplankton (freshwater pelagic endmember) and isopods (freshwater benthic endmember) did not have significantly different $\delta^{34}S$. If $\delta^{34}S$ did not differ significantly between two taxa known to occupy different habitats, it is unlikely that habitat use would differ enough within taxa to explain among-lake variability in $\delta^{34}S$ ratios. High among-lake variation in freshwater endmember $\delta^{34}S$ ratios likely reflects variation in sulfate source.

Sources of sulfate to lakes include the surrounding catchment, atmospheric deposition, and anthropogenic inputs (Wetzel 2001). Atmospheric deposition of sulfate is likely similar among lakes (due to the relatively small spatial scale), and none of the lakes has any significant anthropogenic inputs of sulfate. Underlying geology does differ among lakes, but although Hovaktok and Nauyuk lakes have the most similar underlying geology, they also show the greatest differen-

Fig. 2. (*a*, *b*) $\delta^{34}S$ and (*c*, *d*) $\delta^{13}C$ ratios in endmembers and (*a*, *c*) anadromous and resident Arctic char (*Salvelinus alpinus*) and (*b*, *d*) lake trout (*Salvelinus namaycush*). Plots include ratios for freshwater benthic prey (isopods; shaded squares), freshwater pelagic prey (zooplankton; shaded triangles), marine prey (forage fish; open diamonds), and anadromous (solid circles) and resident fishes (open circles). Points for marine prey and anadromous Arctic char are coincident in (*a*), and points for freshwater endmembers and resident lake trout are coincident in (*b*). Marine and freshwater prey items were better separated by $\delta^{34}S$ than by $\delta^{13}C$.

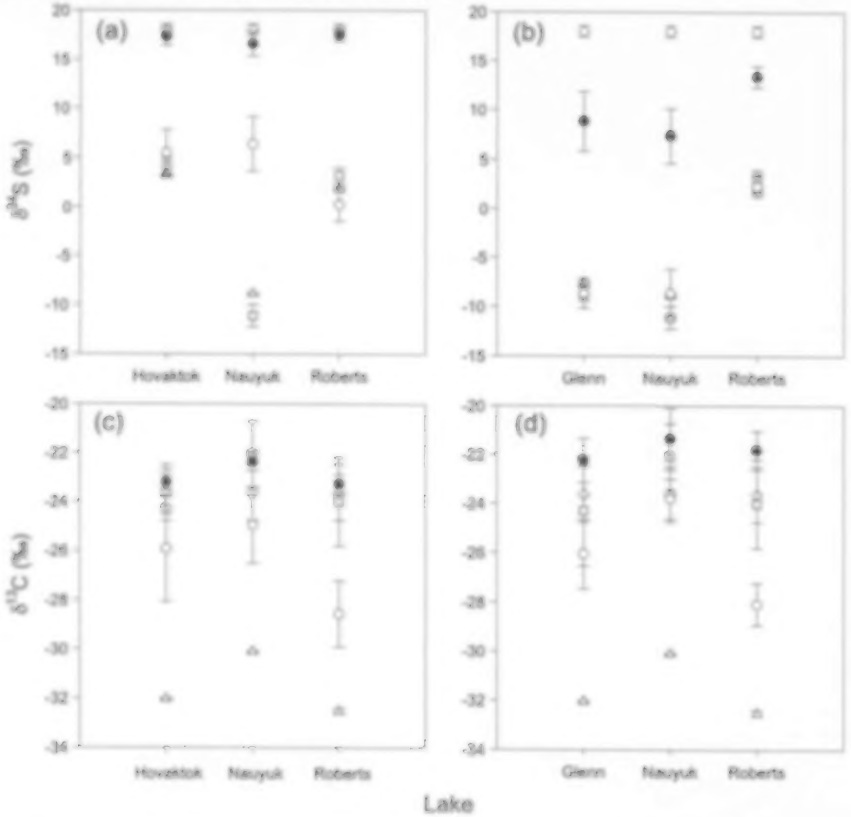


Table 2. Estimates of proportional contributions of food sources to anadromous and resident Arctic char (*Salvelinus alpinus*) and lake trout (*Salvelinus namaycush*).

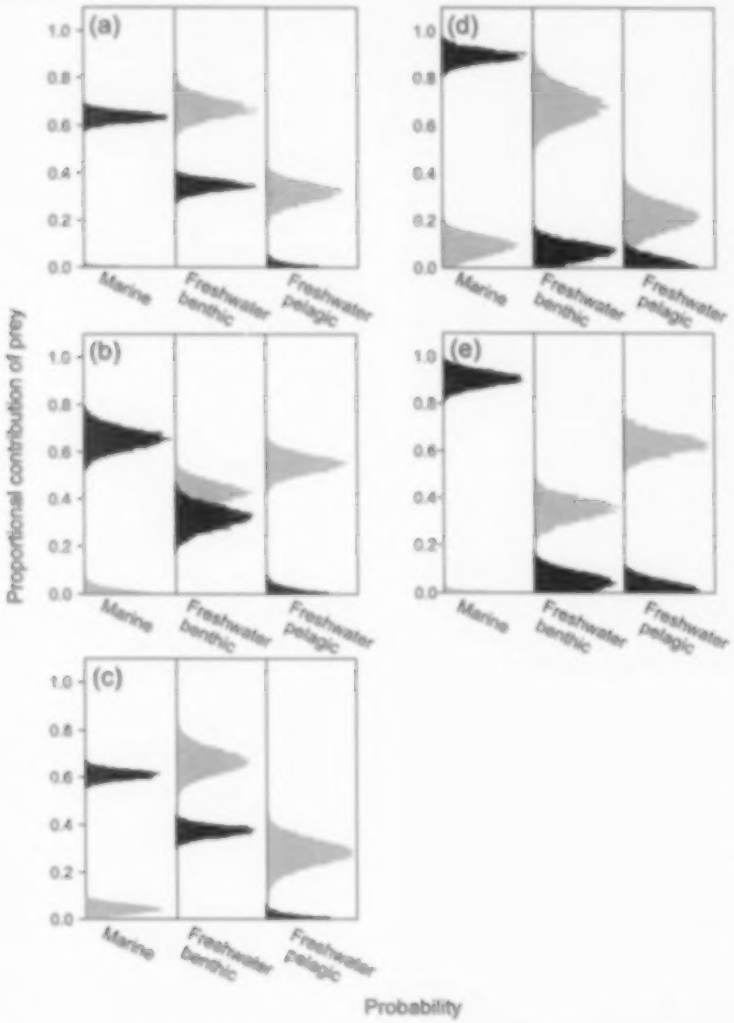
			Marine			Freshwater benthic			Freshwater pelagic		
		N	Lower 95% CI	Median	Upper 95% CI	Lower 95% CI	Median	Upper 95% CI	Lower 95% CI	Median	Upper 95% CI
Hovaktok	Anadromous Arctic char	15	0.85	0.90	0.95	0.02	0.08	0.13	0	0.02	0.07
	Resident Arctic char	3	0.03	0.1	0.16	0.63	0.73	0.85	0.09	0.17	0.25
Roberts	Anadromous Arctic char	9	0.85	0.91	0.96	0	0.05	0.12	0	0.03	0.09
	Resident Arctic char	24	0	0.01	0.02	0.28	0.36	0.43	0.56	0.63	0.71
Glenn	Anadromous lake trout	9	0.60	0.64	0.67	0.31	0.35	0.39	0	0.01	0.04
	Resident lake trout	34	0	0.004	0.02	0.59	0.66	0.73	0.27	0.34	0.41
Nauyuk	Anadromous lake trout	8	0.57	0.60	0.64	0.33	0.38	0.42	0.01	0.01	0.06
	Resident lake trout	15	0.01	0.05	0.08	0.59	0.67	0.76	0.20	0.29	0.37
Roberts	Anadromous lake trout	6	0.58	0.66	0.73	0.24	0.33	0.41	0	0.01	0.05
	Resident lake trout	23	0	0.02	0.05	0.36	0.43	0.50	0.49	0.55	0.62

Note: Arctic char, *Salvelinus alpinus*; lake trout, *Salvelinus namaycush*. CI, confidence interval.

ces in endmember $\delta^{34}S$ ratios (Fraser 1964; Kerr and Knight 1998).

Variation in $\delta^{34}S$ ratios among lakes may reflect variations in Holocene geomorphology. Approximately 9000 years B.P., the study area was deglaciated and inundated by a marine incursion (Kerr 1994; Kerr and Knight 1998). Water and sediments of the study lakes were of marine origin at the time of isolation and by inference had marine $\delta^{34}S$ ratios. Variation in $\delta^{34}S$ ratios among lakes may reflect differences in timing of isolation from marine incursion, relative rates of flushing

Fig. 3. Proportional contributions of marine, freshwater benthic, and freshwater pelagic prey to (*a*, *b*, *c*) anadromous lake trout (*Salvelinus namaycush*) and (*d*, *e*) Arctic char (*Salvelinus alpinus*) in (*a*) Glenn, (*b*, *e*) Roberts, (*c*) Nauyuk, and (*d*) Hovaktok lakes. Anadromous fishes are represented by solid distributions, whereas resident fishes are represented by shaded distributions. Proportional contributions of marine prey were larger for anadromous Arctic char than for anadromous lake trout. (*b*, *e*) Resident fishes in Roberts Lake fed more on freshwater pelagic prey than on freshwater benthic prey. (*a*, *c*, *d*) In other lakes, resident fishes fed more on freshwater benthic prey.



with meteoric waters, differences in depth of marine-origin lake sediments, and differences in concentrations of marine-origin sulfur compounds. Further research is necessary to confirm this. This analysis highlights the importance of determining variation in $\delta^{34}S$ ratios among freshwater systems before applying mixing models. We also suggest that, similar to $\delta^{15}N$, $\delta^{34}S$ ratios may sometimes require baseline correction before statistical analysis.

Differences in isotope ratios between anadromous and resident fishes

Similar to previous studies (Doucett et al.1999; Swanson et al. 2010*b*) and with the exception of Arctic char in Hovaktok Lake, anadromous Arctic char and lake trout had enriched $\delta^{13}C$ and $\delta^{34}S$ ratios compared with resident Arctic char and lake trout. An interspecies comparison revealed that anadromous Arctic char had significantly higher $\delta^{34}S$ ratios than anadromous lake trout. This is consistent with previous, preliminary results presented for Roberts Lake (Swanson et al. 2010*b*).

$\delta^{34}S$ ratios in Arctic char from Nauyuk Lake were unexpectedly high and indicated that resident individuals of this species used a freshwater habitat that was not sampled (i.e., a prey source is missing). Because resident lake trout had similar $\delta^{34}S$ to freshwater endmembers in Nauyuk Lake, the results for resident Arctic char were not due to sampling or laboratory error. They likely reflect a unique migration strategy used by Arctic char in Nauyuk Lake.

Upstream and downstream migrations of anadromous Arctic char were monitored at Nauyuk Lake during the 1970s

Table 3. Estimates of marine source contributions to population productivity.

		Lower 95% CI	Upper 95% CI	Proportion of anadromous individuals[a]	Marine contributions to population productivity (5%–95%)
Hovaktok	Anadromous Arctic char	0.85	0.95	0.46	0.39–0.44
Roberts	Anadromous Arctic char	0.85	0.96	0.36	0.31–0.35
Glenn	Anadromous lake trout	0.60	0.67	0.26	0.16–0.17
Nauyuk	Anadromous lake trout	0.57	0.64	0.40	0.23–0.26
Roberts	Anadromous lake trout	0.58	0.73	0.30	0.17–0.22

Note: Arctic char, *Salvelinus alpinus*; lake trout, *Salvelinus namaycush*. CI, confidence interval of marine food source contribution.

[a]Proportion of individuals that are anadromous in partially anadromous populations of Arctic char and lake trout. These estimates were calculated from data presented in Swanson et al. (2010*b*).

and 1980s by Fisheries and Oceans Canada (Johnson 1989). During the course of this research, no spawning areas for Arctic char were discovered in Nauyuk Lake, and extensive tagging studies revealed that anadromous Arctic char in Nauyuk Lake employ a two-year migration strategy that utilizes Willow Lake, a small, shallow system upstream of Nauyuk Lake, for spawning and rearing. Anadromous Arctic char return from the sea each fall to overwinter in Nauyuk Lake. During the following spring, prespawning adults move upstream to Willow Lake, and spawning occurs that fall. Juveniles hatch and rear in Willow Lake. As smolts, they move through Nauyuk Lake before beginning annual marine migrations (Johnson 1989). High $\delta^{34}S$ ratios observed in resident Arctic char were therefore likely a reflection of prey $\delta^{34}S$ ratios in Willow Lake rather than Nauyuk Lake.

Mixing-model results

It appears that resident Arctic char and lake trout use both benthic and pelagic food sources in the study lakes and that there is among-lake variation in habitat and (or) prey use. Previous research has shown that food webs in unproductive Arctic lakes are often supported more by benthic–littoral production than by pelagic production (e.g., Hecky and Hesslein 1995; Karlsson and Byström 2005). As such, we expected freshwater benthic food sources to contribute more to resident fish diet than freshwater pelagic food sources. This was true for Glenn, Hovaktok, and Nauyuk lakes but not for Roberts Lake. In Roberts Lake, pelagic food sources accounted for approximately 60% of resident Arctic char and lake trout diet. Determining and predicting dominant sources of production in Arctic lakes should be a future research priority in the face of increasing fish habitat alteration and northern industrial development.

Although anadromous Arctic char and lake trout may only use marine habitats for 3–6 weeks each year in the Canadian Arctic (e.g., Johnson 1980), the majority of prey consumed by these fishes is of marine origin. Marine food sources accounted for a larger portion of anadromous Arctic char diet than anadromous lake trout diet. Lake trout have less osmoregulatory capacity than many other salmonids, and until very recently, lake trout were thought to be an obligately freshwater species (see Swanson et al. 2010*b*). It is thus likely that lake trout have lower salinity tolerance than Arctic char and either make shorter marine migrations than Arctic char or migrate to lower salinity habitats than Arctic char. This is consistent with inferences made from otolith microchemistry analyses (Swanson et al. 2010*b*).

Although it is likely that anadromous lake trout utilize marine prey sources to a lesser extent than Arctic char, it is also possible that slower turnover rates in anadromous lake trout resulted in lower $\delta^{34}S$ ratios and lower estimates of marine food source contributions. When organisms switch food sources, the rate of change in isotope ratios depends on tissue turnover rates. Isotopic turnover occurs through both growth and metabolic replacement (e.g., MacAvoy et al. 2001), and tissue turnover rates are faster in fast-growing organisms with high metabolic rates. Anadromous lake trout grow more slowly than anadromous Arctic char (Swanson et al. 2010*b*). Also, anadromous lake trout have more freshwater-derived tissue to replace than Arctic char because they are larger when they begin marine migrations (Swanson et al. 2010*b*).

Stomach content analyses may help to confirm whether anadromous lake trout utilize more freshwater food sources than anadromous Arctic char after onset of marine migrations. Unlike anadromous Arctic char (Rikardsen et al. 2003), sexually mature anadromous lake trout appear to feed in freshwater after returning from marine migrations in fall (H. Swanson, unpublished data). If confirmed in the future, this may be explained by the fact that anadromous lake trout have significantly lower lipid stores than anadromous Arctic char (Swanson et al. 2010*b*). Feeding in freshwater overwinter may thus be an energetic necessity for anadromous lake trout.

Marine prey sources supported between 31% and 44% of partially anadromous Arctic char populations and between 16% and 26% of partially anadromous lake trout populations. These estimates are a starting point and require substantial further investigation. It is likely that importance of marine prey sources varies with lake productivity, marine productivity, ease of marine access for anadromous fishes, and structure of the freshwater fish community. If it had been possible to run mixing models for Arctic char in Nauyuk Lake, marine food sources would likely have accounted for as much as 85% of population productivity. This is because 90% of individuals captured in Nauyuk Lake were anadromous (compared with 46% and 36% in Hovaktok and Roberts lakes, respectively) (Swanson et al. 2010*b*). Uncertainty and among-lake variation in proportion of anadromous fishes is likely high and is virtually unstudied. Estimating these proportions may be further complicated by the fact that fidelity to natal lakes can be low for anadromous Arctic char (this is unstudied in lake trout) (Gyselman 1994). Also, although the presence of true residents has been confirmed in all of the study lakes (see Swanson et al. 2010*b*), it is impossible to distinguish presmolts from residents when fishes are young and small. This likely leads to underestimations of the pro-

portion of anadromous individuals in partially anadromous populations. Although it is not currently possible to determine which juvenile Arctic char and lake trout will become anadromous, it might be possible to determine the proportion of juvenile Arctic char and lake trout that had anadromous mothers; a recent study has shown that $\delta^{34}S$ ratios in otolith cores of *Oncorhynchus nerka* reflect maternal diet and can be used to determine whether fish were born to anadromous sockeye mothers or freshwater kokanee mothers (Godbout et al. 2010).

Arctic char and lake trout are an important subsistence, commercial, and cultural resource in the Canadian Arctic (Van Oostdam et al. 2003), and predicting effects of industrial development and habitat disturbance on these species is of considerable concern to regulators, industry, and northern communities. Partially anadromous fishes such as Arctic char and lake trout show considerable plasticity in life history. This plasticity complicates efforts to quantify use of marine and freshwater habitats and makes it difficult to predict effects of anthropogenic stressors on fish population productivity and persistence. Acquiring better estimates of the proportion (and uncertainty) of anadromous individuals in partially anadromous fish populations should be a priority for future research, along with expanding the spatial scale of the research. Intensive sampling of migrations using two-way fish fences and stable isotope analysis of fish tissues with variable turnover rates could lend insight into timing and duration of marine migrations. Lastly, bioenergetics modeling could complement further mixing-model analysis by providing an alternative method for determining importance of marine food sources to individual fitness and population productivity.

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